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ON THE NATURE OF MALARIA.

MEMOIR BY

PROFESSORS EDWIN KLEBS AND C. TOMMASI-CRUDELI.

REALE ACADEMIA DEI LINCEI (1883—4).

ON THE

ALTERATIONS IN THE RED GLOBULES IN MALARIA INFECTION,

AND

ON THE ORIGIN OF MELANÆMIA.

MEMOIR BY

PROFESSOR ETTORE MARCHIAFAVA AND DR. A. CELLI.

TRANSLATED AND EDITED BY

EDWARD DRUMMOND, M.D., ROME.

PREFACE.

The plan which I have adopted in preparing these Memoirs for publication in England has been, first, to make a careful *verbatim* translation of them; and, secondly, as the amount of space assigned to me in this volume did not allow of their being published *in extenso*, to condense and summarize them, which I believe has been accomplished without obscuring the text, or misrepresenting the meaning, of the Authors.

I have to thank the Authors for their courteous assistance in the study of this subject.

EDWARD DRUMMOND, M.D.

3, Piazza di Spagna, Rome.

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ON THE NATURE OF MALARIA.

CHAPTER I.

PRODUCTION OF MALARIA.—STATEMENT OF RESEARCHES IN THE PAST FOR DETERMINING ITS NATURE.

Endemic malaria has, in the natural and civil history of man, a higher importance than that of all other endemic diseases to which the human race is subject. The latter are met with in more or less limited regions of the globe, but it is, as yet, impossible to define the geographical boundaries of the production of malaria. It reaches its highest point in the tropics, but may be very marked in the temperate zones, and is found even in cold climates. In the northern hemisphere it may originate in all latitudes up to 60° N., and some facts observed in Switzerland by Bergmann tend to show that it may be developed in districts hitherto deemed exempt (the circumstances suggest importation). Hence we cannot at present state what portions of the earth, between the polar circles, may be considered absolutely free. In places liable to it, its production is in inverse ratio to the latitude, and the severity of malarious disease augments in like ratio.

In temperate regions, and still more in the tropics, it may render districts uninhabitable. No other endemic or epidemic is able to do this to the same extent. It constitutes the chief obstacle to the exploration and colonization of the African continent; threatens to reduce to the condition of a desert, vast tracts of the Southern States of America, which were very flourishing so long as they were cultivated by the negro race, which, better than any other, resists its action. In Europe it has desolated whole provinces, populous and flourishing in ancient times. Even where its intensity is not such as to cause

a great annual mortality, it induces a progressive decadence of race, which no other endemic is capable of.

The preservation of society from injurious influences so grave is therefore a socio-economic problem of the first order. The statesmen of all civilized nations have, at all times, striven to get rid of or limit it; and even in semi-civilized Peru, we find water systems, founded and watched by the prudent government of the Incas, in the low lands which skirt the Pacific, in order to increase their fertility and salubrity. In ancient times the Roman rule was pre-eminently distinguished for the vastness and grandeur of the works undertaken to vanquish this public enemy, and such were regarded as among the chief titles of the rulers to honourable recognition by the people. Even in modern times, one of the governments most eminent for civil wisdom and sagacity—that of the two last Grand Dukes of Tuscany—owed its chief claim to renown to the works completed or begun to render salubrious vast malaria-desolated lands. Italy, where malaria has made uninhabitable a great extent of country (chiefly on the western slopes of the Apennines, in Sicily, and in Sardinia), has set herself, since 1870, to the radical solution of this problem, as a great political question. The capital is situated in the centre of one of the most insalubrious regions of the temperate zone, and the soil on which it is built is capable, *in the hot season*, of originating malaria.

The unhealthiness of this season has become greatly less in many parts of the city, owing to the great works of drainage, street construction, &c., carried out during the last nine years, but it exercises, nevertheless, an injurious influence on the development of the city and the public life concentrated there. These conditions of the capital have stimulated research directed to discover the origin of malaria *foci* and the means best suited to limit and extinguish it.

All our knowledge of malarious diseases proves that their true causes must be sought for in the soil of the places chiefly affected, and that the poison, produced in the soil, may be raised to different altitudes by ascending currents of air. *In marshy lands* its production is *nil* or very slight, even when the temperature is very high, *so long as the marsh bottom is separated from the air by a sufficient layer of water.* It grows gradually during the hot season, as the evaporation diminishes this water, and

attains its maximum when a great part of the marsh is uncovered or only separated from the atmosphere by a thin covering of water. There then arises a great quantity of poison which has come to be called by many, even now, *marsh miasm.*

This term ought to be abandoned, because malaria is not developed in all marshy places, and may be abundantly generated in lands which were never marshy. Its production in *non-marshy lands* was supposed to be due to a special chemical and geological constitution—a view chiefly maintained by Heyne and Kirke, but it is untenable.

It originates in soils of very different composition, whilst soils of the same composition, and placed under the same physical conditions, sometimes do, and sometimes do not, produce it. This shows that its development is not bound up with any special chemical composition of the soil, but is insufficient to exclude that, other things being equal, its development may be, more or less, influenced by some chemical quality in the soil. It is not improbable, though it is not proved, that judicious cultivation may so modify the chemical composition of the soil as to diminish its aptitude for originating malaria.

Moreover, the experiments made by Lanzi and Terrigi in 1873,* and repeated by them on a large scale in successive years during the works at the Colosseum,† render it very probable that the addition of lime and its soluble salts to some malaria-producing soils diminishes or suspends this production. It is desirable that such experiments should be made in all malarious soils.

Hygienic researches have hitherto been directed to the physical conditions which favour malaria production in non-marshy lands. All these contain a notable quantity of water in the rainy season, either from their low level or the slight permeability of the subsoil which underlies them. During the hot season this production may take place in the upper strata of the soil when these remain sufficiently damp, as happens in malarious lands covered with woods. If, on the other hand, the ground is uncovered, the summer evaporation may entirely dry the superficial, but does not ordinarily go on to the exhaustion of the lower, strata, which may preserve a remarkable degree of humidity till the return of the next wet season.

* Trans. 11th Congress of Ital. Scient. : Rome, 1873.

† Trans. Academy of Medicine : Rome, 1879.

Malaria cannot originate in these superficial strata, remaining dry, but does so in the wet underlying ones, whenever the air is able to gain access to them through fissures or the porosity of surface soil, or when excavations lay them bare.

In low lands these subterranean foci are sometimes formed by the old marsh bottoms covered by natural or artificial *colmate** (such as, for example, the *cuora*† of many places on the coast or Tuscan Maremma, and the subsoil of most of the Val di Chiana). The subterranean marshes are harmless even when they remain wet during the hot season, if the soil of the *colmata* covering them is deep and sufficiently compact. On the other hand, they generate malaria when only separated from the air by a thin stratum of disintegrated soil,‡ or when the air can reach them owing to excavations or fissures in the overlying soil.

Often, however, subterranean *foci* have no marsh origin, are poor in organic *detritus*, and are found at high altitudes of mountains. The preservation of humidity in lower strata of this sort of soil is chiefly due to the sparing permeability of their subsoil and the irregular inclination of its surface impeding the escape of water. Such are frequent in Italy, and especially in the Roman Campagna and City of Rome, as may be seen by examining a section plan of the aquiferous zone from the Janiculan to the Porta S. Lorenzo past the Pantheon. In such soils malaria is not generated unless the access of air to them is brought about by excavations and other such means. Other hypotheses have been started unconnected with these conditions of soil, and have become sometimes popular traditions. One of the most common is that which attributes to forests the power of generating malaria from the decomposition of the leaves and dead branches which strew the ground. This has been suggested by the existence of many forests in Europe, Africa, India, &c., &c., in which malaria attains great proportions; and because disafforesting has had the effect of rendering many such districts salubrious.

Even in the Pontine marshes an analogous fact was witnessed after the clearing made twenty years ago in the great wood

* *Colmata*, embankment, levelling up of hollow places with earth.

† *Cuora*, equivalent to bog or turf; the carbonized black ooze of marshes resulting from the decay of successive generations of organic materials; a term also used for marsh conduits.

‡ Dr. G. Lindi, Paper read before the Medical Congress at Arezzo, July 10, 1877.

which surrounds the Cisterna country on the south. This wood is that of which Lancisi prevented the felling in 1714, being persuaded that woods ameliorated the air of marshes, and in this case protected Cisterna from the south wind which he believed conveyed the malaria.

On the contrary, the destruction of this great wood has procured for Cisterna a marked increase of salubrity and population.* In spite of this, the explanation is fallacious. The existence of decomposing vegetable *detritus* in a forest is not sufficient of itself to cause malaria, as we see in other parts of the globe vast forests, containing such detritus, which are, notwithstanding, salubrious, as, for example, some virgin forests of South America. It is, however, certain that when woods cover malarious lands, they may favour its production, especially if situated at a level which hinders the outflow of water.

The wood, intercepting the sun's rays, impedes evaporation and allows the malarious soil to retain much moisture during the hot season, *even in the strata most exposed to the action of the atmosphere.*

This obstacle being removed, the consequent summer desiccation markedly diminishes the malaria production, and if the soil be not deep may go so far as to arrest it. One can understand, therefore, how disafforesting alone may increase the salubrity of such places, and how, on the other hand, in deep soils, it cannot arrest malaria without the help of such drainage as may exhaust the water of lower strata which the sun's rays are not able to dry.

It is the opinion of many that malaria may be generated by the decomposition of low organisms, animal and vegetable, which die in large numbers when sea water is mixed with fresh water, collected in its vicinity. This opinion is based on the coincidence that in some parts of the Italian coasts where fresh water ponds are only separated from the sea by dunes, intermittent fevers occur.

This has, however, only been met with where the surrounding lands are malarious; or were so formerly, and appear still capable of becoming such again, whenever the old marsh *cuora* which they contain are placed, in summer, under favourable conditions

* Lancisi, *Unedited Addresses on the Cutting of the Woods of Cisterna and Semoneta.*

of moisture and exposure to atmospheric air. Besides, this coincidence is not observed except in seasons abundantly malarious. At other seasons of the year this mixture occurs so as to determine the death of a very great quantity of animal and vegetable organisms, rendering turbid and fœtid the waters of these coast lakes (*e.g.*, Burano, Fogliano, dei Monaci, Caprolace), without fever showing itself even amongst those who spend whole days in fishing and shooting on them. If, then, the lands adjoining be not malarious, the mixture of salt and fresh water may assume vast proportions without the malaria appearing, as is seen in large littoral lakes of the Baltic (Frisches Haff and Curisches Haff), which do not generate malaria, while it is produced in localities identical as to latitude and climate (Jahdebusen and Wilhelmshafen), which, however—a difference of the first importance—are surrounded by malarious lands.

If, therefore, we have not sufficient evidence to exclude that this mixture may aggravate the unhealthiness of a place, we have enough to show that it cannot, of itself, generate malaria in a healthy locality. The same holds true of the opinion that steeping hemp and flax may evolve malaria. Such become offensive from the putrid effluvia they give off, and from contaminating drinking water, but they do not cause malaria.

Such macerations are largely practised in districts which are not malarious and do not originate fevers in them. This does not prevent the possibility that, when practised in malarious localities, they may aggravate the evil, if in no other way, by the excavations necessary in making the tanks, by which air may be admitted to otherwise harmless infection foci. Certainly every reason of prudence counsels the removal of all concomitant causes of unhealthiness, that nothing tending to amelioration may be left untried. But the essential aim of the latter is that of *modifying the true seats of production—viz., the malarious lands—in such a way as to take from them the possibility of offering a field for the development of malaria.*

Experience has shown that this cannot take place without a combination of three factors:

1. A very high temperature.
2. A persistent humidity of the soil.
3. The access of air to the moist strata of the soil.

When one of these is eliminated development of malaria cannot

take place. Even in malarious districts left to themselves, it sometimes happens that, if the mean summer temperature be very high and no rain falls, the production of the poison is arrested, because evaporation from the soil exhausts all the moisture it contains. The occurrence of rain will, however, determine its development in a few hours. So, also, if the summer mean is very low malaria is not developed, or is confined within narrow limits, but it recovers all its force in a very short period if the temperature of the air is raised. The chief aim of works of amelioration up to the present time has been to effect the removal of one of these factors by drying malarious soils. The necessary hydraulic works have varied according to the quality and level of these.

In low-lying lands, to prevent the stagnation of water in the soil and subsoil, the system of open channels has been adopted, in order to secure at all seasons of the year the escape of water. When the land has a level equal, or nearly equal, to that of the sea or natural collectors of the district, this system has been combined with pumping machines, or with the system of *colmate*, wherever it is possible to introduce watercourses rich in suspended soil to the lands to be ameliorated. The rise of level procured by the *colmate* has the twofold advantage of draining the soil and burying that part of it in which the malaria originates, so that, when successful, this secures the removal of two of the factors.

In lands situated at higher levels, measures have been adopted according to the conditions present—open canals, great collecting pipes, superficial drainage by open canals and earthen pipes, and even drains constructed at a remarkable depth. The most beautiful examples of these last are afforded by the *cunicular* drains, met with in the Roman hills and Agro Romano, where, according to Lanciani, this system reached its highest development in the imperial epoch.*

These methods remove one or two of the external conditions indispensable to malaria production, but do not destroy that special tendency of malarious lands which constitutes the essential difference between them and those which, identical as to chemical composition and surrounding conditions, are incapable

* Lanciani, *Some Works of Amelioration of the Agro Romano, executed by the Ancients.* Also Secchi, di Tucci and others.

of originating this poison. The cause is simply reduced to impotence; but examples are not rare in which, after this has happened, the development has recommenced and attained large proportions when the external conditions, which had been banished, were reproduced. In a district long free from malaria, neglect of the water scheme, inundations, or superabundance of rain, or accidental opening up to the air of some of the old *foci*, suffices to start the malaria again.

The conversion of the Crown domains of the Val di Chiana into private property has led, in a great part of the valley, to neglect of the hydraulic works, allowing a return to the former state, and consequent reappearance of malaria in many places in which there had long been no trace of it.

It is this which has happened in the Agro Romano, which in the imperial epoch had been rendered so healthy as to permit of a summer residence in villas situated in spots now regarded as amongst the most insalubrious in the temperate zone, showing that not even centuries of exemption can ensure that the cause has been got rid of, beyond the possibility of recurrence. This remarkable tendency of malarious lands has stimulated research as to the nature of the agent that produces this effect, in the hope that, this being once known, means might be discovered of destroying it and rendering its development impossible, and thus obtaining a lasting and secure amelioration. The first researches were directed to the examination of the gases diffused in the soil for one capable of generating it, but with only negative results. Again, the supposition that some volatile product of animal or vegetable decomposition might be the cause has not been borne out by facts.

It has never been shown that the mud and air of marshes contain an inorganic substance capable of developing, without the intervention of any other substance, malarious fever.

Moreover, its production, even in marshy lands, is sometimes wanting, although animal and vegetable organisms undergo putrefaction as actively as in those which are malarious; while, on the other hand, malaria may develop in non-marshy lands in which such putrefaction does not occur.

The want of exact correspondence between the quality of the soil and the production of malaria has led some to suppose that such may not be due to a specific cause, but rather the result of

sudden or very rapid lowering of temperature, rendered more injurious by the marked humidity generally found in malarious countries. This opinion has been adopted by even distinguished observers like Santarelli,* and sustained recently by Oldham.† Here the essence of the disease has clearly been confounded with a frequent, but not invariable, cause, *determining development.*

Undoubtedly many persons, so long as they use every precaution against chill, live with impunity in malarious countries, but are seized with an attack if they leave off such precaution, and this even after having left the malarious district.

The same holds good of cholera and other like diseases, and is easily explained by the disturbances of the circulation which chilling brings about in vascular areas, which allow the disease-ferment, already in the system, to fix itself in some part of the body and there display its evil influence. Besides, malarious fevers may arise without the intervention of this cause, as one may easily satisfy himself, especially in places where the graver forms of malaria prevail. On the other hand, in *non-malarious* countries, this cause (chilling), however severe it may be, never originates a malarious fever.

The same reason—viz., the inconstancy of the relation between condition of soil and malaria production—which has led some to reject the idea of a specific cause of infection, has suggested to others that malaria may be due to a parasitic organism which requires for its development not only favourable external conditions, but also the presence of a germ capable of giving rise to it.

Various reasons tell in favour of this hypothesis; among others, certain analogies which are common to malaria and other known parasitic infections, and the augmented production of the former when left to itself. When man's industry ceases to remove the external favouring conditions of malarious production, this assumes such vast proportions as are inconceivable without the existence of an organism capable, when its evolution is no longer checked, of infinite multiplication. This enormous increase is historically proved, especially in Italy.

The Tuscan and Roman Maremma, the Roman Campagna, Pesto, Selinute, had malaria in ancient times, but were inhabited by numerous and prosperous populations; now they are hardly

* Santarelli, *Researches.* † Oldham.

habitable except where man's industry has again been applied to remove the conditions on which malaria depends.

The conception of a living organism which, finding no check to its development, has the power of extension by an infinite number of progressively increasing generations, seems to fit best the magnitude of the phenomena.

The diffusion of malaria, in the air overlying lands which produce it, furnishes some arguments in support of the hypothesis that it is due to the agency of solid particles.

The first hours of morning, and still more of evening, are the most perilous to those who breathe this air; besides, experience has shown that the cause to which this insalubrity is due is often confined to the lower strata of the air, and at a few metres from the ground ceases or notably diminishes. In the Pontine marshes, whilst the effect of sleeping during a summer night, so as to breathe the air nearest the ground, would inevitably cause an attack of perniciosa, people sleep with impunity on a platform raised on piles a few metres from the ground. In South America people similarly circumstanced preserve themselves by sleeping in hammocks suspended from the trees. This is explicable on the supposition that malaria is due to solid particles of slight specific gravity contained in the soil, and raised to a certain altitude during those hours in which the difference of temperature, in the lower strata of air and in the soil, causes an upward current; and that this does not happen, or only in a minor degree, when the two temperatures are almost equal. Were the agent a gaseous one due to chemical action, it should be at its acme in the day hours, when the soil is most heated by the sun's rays.

Not less does the hypothesis that malaria may be due to a volatile substance united with watery vapour, raised with it in the atmosphere during the day and reconducted back to the earth at sunset, afford an explanation of that immunity which is enjoyed at a few metres from the soil.

Lastly, the mists which form at evening in malarious places, and which are often fœtid, from the products of putrefaction they contain, are not confined to the strata nearest the ground.

They may be very thick even at high altitudes, so that in the plains and valleys they appear, in the early dawn, when the sun has not yet appeared above the horizon, like a sea which covers and conceals all, even the church towers.

These considerations, therefore, if they give some probability to the hypothesis of the parasitic origin of malaria, are certainly insufficient to convert it into a scientific theory.

In addition to this it is necessary to show—1*st*. *The constant presence of a determinate organic species in the various kinds of malarious soils and in the air which overlies the same.* 2*nd*. *That this organic species can alone—viz., without the intervention of any other morbific agent—generate a true specific intermittent fever.*

This double demonstration, indispensable to the theory, has not yet been obtained, although for thirteen years researches have been instituted with this object.

In 1866 Salisbury believed he had discovered the cause of malaria in an alga of the genus *Palmella*, which he had found in certain marshes in Ohio.*

He maintained that the spores of this alga, diffused in the air and penetrating within the human organism, produced malaria. In support of this opinion he adduced the fact, that having placed some boxes of earth containing this alga on the window-sill of the room in which slept two young men with the window open, both took intermittent fever in fourteen days. Their dwelling was five miles from the spots whence the earth was taken, but it was not certain that they might not have visited these places and others equally unhealthy.

But granted that the fever in these two persons was due to the earth contained in the boxes, one such experiment was insufficient to prove that it was due to the *Palmella gemiasma* of Salisbury, and not to other organisms contained in the same.

Later, this *Palmella gemiasma* has been found in very healthy places, and even on the Alps, and Lanzi and Terrigi have seldom found it in the stagnant waters of the Campagna, and have never met with its spores in the air of the most malarious places examined by them.

In 1869 Dr. P. Balestra described an alga found by him in the Pontine marshes and in those of Ostia and Maccarese, which he held was the cause of malaria. He described neither its genus nor species; however, Lanzi and Terrigi decided to regard it as *Cladophora* or *Œdogonium*, which abound in these places. In a later edition (1877) Dr. Balestra, without yet determining the

* *American Journal of Medical Sciences*, 1866.

plant, still less stating its exact dimensions, gave two figures of it entitled *Alga miasmatica*, so shapeless that one cannot tell what it was meant for—an alga, a fungus, or some other organism.

The experiments made by Balestra to prove that the plant studied by him was febrigenous do not avail to prove it. He treated some fluids containing it with quinine, arsenious acid, and sulphite of soda in solution, and found that they, especially the quinine, arrested its growth and altered its structure.

It is insufficient to establish such relation between this plant and quinine, as Binz proved long ago that the latter kills a large number of animal and vegetable organisms which certainly have no influence on malaria. In Balestra's experiments the quinine also killed all the infusoria and suppressed putrefaction for a very long time. This author has not sought to show the specific action of this plant by isolating it and procuring intermittent fever by introducing it into the organism. The only evidence of this kind adduced by him is that he was himself seized with intermittent fever, eight hours after having accidentally drunk from a carafe containing marsh mud, on the surface of which a stratum of this plant had formed. When we remember that this happened when he was working during the height of summer in Rome, and making excursions to the Pontine marshes, Ostia, and Maccarese to collect material for study, we cannot but think that the fever might have had another origin; and however ready to admit that it was due to the contents of the carafe, there is no proof that the cause was this particular plant, and not an inorganic substance or one of the numerous other organisms contained in marsh mud. His drawings show this variety of organisms, and along with sporules of this alga, many others besides—small granules like micrococci of Schistomycetes, and also larger bodies and filaments like the germs of Hyphomycetes. Probably the author did not attach any importance to these, as he still believes in the polymorphism of Hallier, and holds that these organisms may pass from the one form to the other without change of nature; but now for many years the works of De Bary, F. Cohn, Naegeli and others have shown the insufficiency of this convenient hypothesis. It is to be noticed finally that Balestra does not appear to have found the plant he believed to be febrigenous in the non-marshy malarious lands of this province.

Safford and Bartlett believed they had discovered the cause of malaria in *Hydrogastrum granulatum*, Archer in *Chtonoblastus aeruginosus*, and Bargellini in *Palmoglea micrococca*, simply because each had found in abundance the plant which arrested his attention in the marshy soil taken for examination; but these three are found abundantly in all soils rich in moisture, while Lanzi and Terrigi have rarely met with them in the soil of the Roman Campagna; besides, these authors observe that the spores of the *Hydrogastrum g.* and *Chtonoblastus a.* are of larger diameter than the capillaries, and it is therefore impossible for them to enter the circulation and cause fever.

Griffini, in 1873, performed experiments on dogs and rabbits with dew collected on the marshes and rice-fields.

This dew contained a *bacteria*, *vibriones*, some filaments of *leptothrix*, some *spirillum*, and many infusoria of the genera *Monas* and *Cercomonas*.

Injecting it in quantities from 75—100 cc. into the veins of dogs, he obtained after the first injection augmentations of temperature of 0°·6—1°·5 C., which lasted a short time and gradually passed away.

The injection of 100 cc. of water from a rice-field, taken July 16th, into the jugular vein of a dog, produced a temporary rise of 0°·6 C., whilst the injection of 96 cc. into the jugular vein of another dog produced a rise of 0°·5, which lasted three hours and then passed away. In rabbits the injection of dew into the jugular (8—10 cc.) caused the death of the animals after 5—12 hours, without notable rise of temperature. A rabbit, under the skin of which 2 cc. of the same dew was injected, died in 28 hours without having shown any rise, but, on the contrary, a progressive fall, of temperature. A rabbit which was made to swallow 20 grammes of dew, and had 5 grammes more injected *per rectum*, showed from the beginning a rise of 0°·6, but afterwards the temperature fell 2°·5 C. On the second day it died. In none of these animals in which the injection produced a rise of temperature did there follow a second access of fever, and the measurements were too few to determine the type of fever. The spleens were normal, but do not appear to have been examined microscopically. The blood showed no increase of the organisms contained in the dew.

Drs. Lanzi and Terrigi, of Rome, applied themselves to the

study of the subject in 1870, and communicated the results of their first observations to the Medical Section of the 11th Congress of Italian Scientists, October 19th, 1873.

At that time Dr. Matteo Lanzi had by a series of artificial cultivations, instituted with mud collected in certain malarious spots in the City of Rome, and the salt marshes of Ostia and the Pontine marshes, obtained the specimen called *Monilia penicellata*, Fr. (*Briarea elegans*, Corda), and was disposed to consider it the cause of malaria. Later on, however, continuing his researches with the conscientious assiduity which characterizes him, he obtained so many organic species as did not permit him to retain his first opinion, so that he and his colleague Terrigi, fearing to be misled by fallacious appearances, abandoned the parasitic theory of malaria which they had sustained in 1873, and admitted instead that malaria consisted in a *cadaveric vegetable product* generated by putrefaction of algæ and other plants. They undertook, therefore, new experiments with mud from the marshes of Ostia. The hypodermic injection of dogs during winter with Ostia mud (collected during the previous summer) gave rise only to passing disturbances, and in the bodies of the animals no *post-mortem* evidence of malaria was found. The simultaneous injection of guinea-pigs with the same material produced some ill-defined morbid states, and in one of the animals was found, in the spleen and liver, some black pigment. The temperature does not seem to have been taken in the first experiments. The experiments were repeated in the height of summer with mud (August) from the Stagni of Ostia. This was injected hypodermically into two animals—the observers having first ascertained by a few autopsies on healthy guinea-pigs that the blood contained no organisms and the spleen and liver no black pigment.

The first of the two died in 57 hours, after a rise of temperature which reached 40° C., and then fell to 34° and ultimately to 31° C. (axilla). *Autopsy.*—Many bacteria at injection site, spleen and liver enlarged, and containing small quantity of black pigment; a few granules also in the blood of the portal vein.

The second animal died on the thirty-second day; maximum temperature, 38° C.; minimum, 37° C. An abscess, which opened spontaneously on the twenty-second day, formed at the site of injection. *Autopsy.*—Spleen and liver enlarged. Granules of pigment in the spleen and blood of the portal vein. The same

results, with the sole difference of greater amount of pigment, were found in guinea-pigs made to breathe for many hours, in a confined atmosphere, the effluvia from Ostia mud, and in the case of one to breathe like effluvia from decaying grasses collected in August outside the Lateran gate. Parallel to *each* of these experiments another guinea-pig was kept in an equally confined atmosphere containing no effluvia for a like number of hours. None of the animals, in this counter-experiment, showed any signs of pathological disturbances. Each was killed at the same moment as the corresponding animal experimented on, and no trace was found of the alterations met with in those which breathed infected effluvia. Lanzi and Terrigi* have the merit of having used a more rigorous method than any of their predecessors, and of having taken the first step towards the solution of the problem; and the discovery of black pigment shows that *they first* succeeded in procuring malarious infection in animals. As, however, they did not examine the splenic swelling, ascertain the proportionate weight of the spleen to the entire body, or make sufficient thermometric observations to determine the type of fever, the observations are inadequate. Nor did they ascertain if the black pigment contained iron in inorganic combination, which distinguishes the *melanæmic* pigment of malaria from other black pigments (melanin).

It is therefore uncertain whether the fevers were really malarious or were produced by septic infection, all the more as the materials used were rich in putrefying organic matter.

As regards the nature of malaria, these talented observers have only expressed the opinion that its cause is a *cadaveric vegetable poison*—a non-living ferment—but they have adduced no proof, direct or indirect, in support of their opinion; they have not isolated the substance and shown that it can generate malaria; nor were they certain before commencing the experiments that all the organisms were dead.

After the meeting of naturalists at Cassel, September, 1878, we undertook the study of this question in the Roman Campagna in the spring of the following year, and one of us (Tommasi-Crudeli) set himself to study and define the foci of malaria in the extent of the Agro Romano in the course of the winter.

* Lanzi and Terrigi "On Vegetable Miasm, Malaria, and the Climate of Rome," Acad. Med., Rome, 1876.

CHAPTER II.

METHOD OF RESEARCH.

To achieve the aim of our researches, it was necessary that they should take into account all existing opinions respecting mycotic, and the special peculiarities of malarious, disease; and that these should be dealt with by such rigorous method as should enable us to avoid the *petitio principii* into which some of our predecessors had been led. We had to decide what symptoms and morbid appearances should be regarded as characteristic and establishing the identity of malarious disease artificially produced. This presented little difficulty, as regular intermittency of febrile attacks, enlargement of the spleen, and the special anatomical characters of malarious splenic hyperplasia are amply sufficient for the purpose.

Omitting the so-called "larval forms," we have to consider the febrile states of continuous course which are seen alone or in combination or alternation with the more regular intermittent forms, such as the form of malarious fever seen in Italy, to which has been given somewhat loosely the term "Roman fever." Such differ so materially in their clinical aspects from ordinary intermittents, that some have believed them to be non-malarious, an opinion long since refuted by clinical experience, or as due simply to chill, of which the fallacy was shown in the last chapter.

The opinion that malarious is convertible into typhoid fever is one of which no proof has been adduced, and while it is most improbable that one disease should be converted into another, it is not improbable that the two may sometimes be united and run parallel in the same individual, although this has not yet been demonstrated by an autopsy.

That an intermittent may be converted into a continuous or subcontinuous malaria fever, a fact long known in human pathology, has been shown, by our experiments, to happen in the

artificial malaria of animals.* *A priori*, artificial production of malaria in the latter would seem unlikely, as in malarious districts most deadly to man they appear to enjoy a perfect immunity; and certain diseases and poisons which attack man seem not to affect animals, and *vice versâ*.

Experimental pathology has shown, however, that such immunity is more relative than absolute; and the tubercular and diphtheritic infection have been produced in animals, while one of us (Klebs) has shown that syphilis may be communicated to monkeys.

Contrasting the unhappy condition of man in, for example, the Pontine marshes, with the thriving state of thousands of mammals, there exposed much more completely to malarious infections, it is difficult to explain the immunity of the latter.

The rule, however, seems to have its exceptions, as certain breeds of horses introduced into the Campagna are said to suffer from enlarged spleens; and flocks of goats are said to be decimated in such localities by disease attended with splenic enlargement. Some part of the immunity of animals may be due to acclimatization, just as the negro race resists as no other can the malarious poison, and thrives in tropical lands uninhabitable by the less resistant white races. This may in part be the outcome of natural selection in the hereditary struggle against malaria aggression, and in the long course of centuries animals may acquire a power of resistance to malaria so long as it only reaches them through accustomed channels. The greater extent and tortuosity of the nasal respiratory passages, and the necessity for air laden with malaria germs to traverse such an extent of moist membrane, may, in animals, place them at an advantage in comparison with man, whose respiration, unlike theirs, is chiefly buccal. Experiments might settle this question, but it only falls within our design to secure the introduction, into the circulation, of malaria poison, and to observe if a true malaria fever results. We confined ourselves to the subcutaneous injection of the suspected material, and obtained the positive results set forth in the next chapter.

In order to judge of the precise nature of the fever so origi-

* My experience coincides with that of Sir Joseph Fayrer and others, that a fever, clinically indistinguishable from typhoid, arises in malarious countries, from a cause other than fœcal emanations.—E. D.

nated it is necessary that the temperature should be taken very accurately and often. We, in our experiments, had it taken every two hours from 6 A.M. to 10 P.M., and sometimes also at midnight, at first using a maximum centigrade (Geissler), and when this was broken, a good Roman one—both by a curious coincidence, indicate an error of —0°·1, as ascertained by our colleague Cannizzaro. *In our tables and temperature curves this error requires to be corrected.*

The weight of the animals was carefully ascertained before commencing the experiments and during their course. At the beginning of malarious fevers, especially the intermittent, there is slight increase of weight; but later on, and particularly in the continuous forms, there is loss, and often rapid loss, of weight. *This point is of very great importance.*

In malarious infection naturally occurring in man and artificially produced in animals there is enlargement of the spleen, from hyperplastic tumefaction, with *uniform increase* of *all* its dimensions without loss of sharpness in its angles and borders. In septicæmic enlargement the organ is uniformly rounded and altered in sectional outline. The method which we adopted to record the size of the spleen was a species of "*nature printing*." The convex surface of the spleen was smeared with blood and applied to sized paper to obtain an accurate print; afterwards the transverse section was so taken. In this way we obtained the figures in Table I.

Another characteristic of malarious affection is the production of black pigment of hæmic origin, which is not only found in perniciosa, but in milder and more chronic intermittents. In severe forms it is produced in great abundance, and is met with in the blood, spleen, and bone marrow. Unlike *melanin*, it contains iron in inorganic combination, and treated with hydrochloric acid and ferrocyanide of potassium produces Prussian blue. Its derivation direct from the blood is now ascertained. No like change of hæmoglobin during life is met with in any other disease (with the exception perhaps of Melanosarcoma), and its presence is therefore a sure evidence of naturally or artificially produced malaria.

Once satisfied of the identity of maladies artificially produced in animals by the injection of a crude material with those resulting from spontaneous malarious infection in man, it becomes

necessary to separate from this material the true excitor of disease. Assuming that this infection, like some others of which the etiology is now well known, may be due to animal parasites, its isolation becomes possible. In this material it is presumable that, besides the true excitors of the disease, there are other substances which do not contribute to this result. There may be living organisms or inorganic matter.

We are not concerned with the latter, whose development should encounter more difficulty as conditions are supplied favouring the growth of the former. We know the three conditions (Chapter I.) experience has shown to be indispensable to malaria development. We reproduced these conditions in the following way. In a large well-ventilated air bath were placed on wooden shelves, above a layer of sand heated by gas, some large flat capsules of porcelain. Some of these were filled to the height of 5 cm. with earth kept constantly wet. Others contained a layer of water, renewed as it evaporated, and within which were placed some small tin boxes full of earth. The sides of these were perforated a little way from the bottom, so as to allow the water to penetrate and keep constantly moist the lower strata of the earth. Thermometers were placed so as to show the temperature of the air and earth, and during the entire day a temperature of 30°—35° C. was kept up. In the evening the gas was extinguished, and the air bath and earth in the boxes remained at the same temperature as the air of the laboratory.

In this way we reproduced, in these *artificial* marshes, similar to those of the Campagna, all the conditions of temperature, moisture, and access of air which are found in malarious lands at the time of their greatest activity.

The most notable result was that almost immediately a large number of the organisms in the earth and mud died. The development of diatoms and desmideæ and also of hypomycetæ was suspended. Moulds ceased to form on the surface of the earth and vases, the phenomena of putrefaction were arrested in the marsh mud, whilst they attained a high degree when this was kept under a bell-glass. The elimination of a great part of the organisms in the crude material rendered easy the search for those which are concerned in causing malaria. In this we employed the method devised by one of us (Klebs)—called by him "fractional cultivation," afterwards adopted by Pasteur, and by

means of which he has procured the isolation of various pathogenetic organisms.

When from a liquid containing a not too great variety of organisms one takes a single drop and places it in a cultivation fluid, if this is fit to favour the development of such organisms, these develop in greater number, which are most numerous in the original drop. Ordinarily this preponderance is found in those organisms to which is due the morbigenous energy of the original fluid. If not, the basis of culture and atmospheric conditions are so altered as to result in killing the pathologically inactive organisms, or at least to oppose such obstacles to their development as allow the pathogenetic ones to gain the supremacy. If this happens in one cultivation only, it becomes easy, by repeating the operation, to separate the sparsely developed organisms from the pathogenetic ones, which are always developed more abundantly, and at last obtain a cultivation of these to the exclusion of all others.

If the experiments made with the pure culture show that it excites the same morbid processes as the crude material, it then remains to ascertain if these are due to the liquid basis of cultivation or to the organisms which it contains—probably the latter, as the former is undergoing a geometrically progressive dilution.

To obtain direct proof of the exclusive pathogenetic efficacy of the organisms cultivated, it is necessary to ascertain, by direct experiment, the impotency of the liquid part of the final cultivation.

This is done by filtration through cylinders of potter's clay or filters of plaster of Paris by means of the air-pump (Klebs, Zahn, Pasteur), or even by passing through good filtering paper. The use of this last method is certainly more simple and convenient, but insufficient when one has to do with very minute organisms, as those of septicæmia, diphtheria, rheumatism, and the like; but in the case of malaria we have been able to obtain sometimes by its means a progressive diminution of the efficacy of the filtered liquid. *Since all the fluid parts traverse without obstacle the pores of the filter, one obtains even in this way the proof that the solid particles, retained wholly or for the most part on the filter, are the holders of the pathogenic activity displayed by the cultivation, taken as a whole.*

CHAPTER III.

RESEARCHES.

For the sake of brevity I have introduced the following contractions into the chapter, more particularly in recording the experiments :—

temp....	temperature cent.	L.	length.	W.	weight.
max. ...	maximum.	B.	breadth.	I. S......	index of spleen.
min. ...	minimum.	T.	thickness.	F. A. ...	febrile *accès*.

Our researches, being occupied with the solution of the general question, were also directed to the special one of malaria in the Roman Campagna, and it became necessary to select a locality in which, beyond doubt, the gravest forms of the malady are seen in the hot season. The Pontine marshes offered the most suitable conditions, especially as we were able to avail ourselves of the liberal help of the Prince of Teano (Onorato Caetani). Indeed, Cisterna, the central point of the property of the Caetani family, enabled us to extend our observations, on the one side to Ninfa, at the foot of Monte Lepini, and on the other as far as the coast of the Tyrrhene Sea, in the district of the lakes of Fogliano, dei Monaci, and Caprolace.

Although malarious fevers had not yet appeared in these districts at the time of our visit, we determined to examine the air of the same. For this purpose we provided ourselves with a ventilator devised by one of us (Klebs) and made by Herr Rothe, Mechanician to the Physical Institute, Prague.

It consisted of a brass box, elliptical on section, in the centre of which revolved an axle, which sustained four metallic wings, fitted to the cavity of the box and slightly curved.

The aperture for entrance of air is at the side, circular, diameter 2·5 cc. In order to take air from any given point, even at a distance, one can elongate the short metallic tube attached to this aperture, by affixing slender tubes of gutta-percha, united together by caoutchouc tubes. The air issues from the box by means of an aperture of the diameter of a centimetre, to which is fitted a metallic tube projecting beyond the apparatus, and to which can be applied means for collecting the air, either tubes containing liquids or glass slides smeared with gelatine or gly-

cerine to fix the solid particles which impinge upon them. The interior and the fans are kept perfectly clean, because the box is formed of two symmetrical halves united by means of screws. The rotation of the ventilator is secured by clockwork set in motion by two bilateral handles. By its agency one can aspirate 300 litres of air in 16 or 17 minutes, by 50,000 revolutions of the axle, recorded by a register.

We believe this instrument to be the most efficient, superior to the apparatus used by Lewis and Cunningham and to the aspirator of Pouchet. Direct microscopic examination of the air of towns did not yield much result (although we did not neglect to make such in Rome), because of the smallness of the micrococci and their mixture with so many other objects. We attach much greater importance to the "cultivation" of such material, because by its means we obtained some of our most certain results, which prove that "*in malarious districts the poison is raised above the level of the ground, even before the time when the infection becomes manifested in man.*"

Previous to our excursions into the Pontine marshes we collected in Rome four samples of air; and April 9th, one at Ninfa (No. 5); April 10th, one at Tre Ponti, on the Appian Way (No. 6); and on 11th five at the coast lakes (Nos. 7—11). For each sample were employed 300 litres of air from near the ground, in Ninfa from land covered with vegetation, near a ruined church; at Tre Ponti from marshy land; No. 7 from the lake of Fogliano, near to a dune, in a spot exhaling a fœtid odour. The temp. of the water of the lake was 15° at 9.15 A.M.; that of the air, 15°·75. At noon, after rain, the temp. of the water was in the *Fossa papale*, which unites the lakes dei Monaci and Caprolace, 16°·5 and 16°·17; that of the air, 18°·2. Samples Nos. 8 and 9 were taken on the lake of Caprolace between 1 and 2 P.M.; Nos. 10 and 11 on the east bank of the same lake between 2 and 3 P.M. At 4 P.M. the temp. of the water of the *Fossa papale* was 17°; of the air, 17°·6. At 5 P.M., lake of Monaci, temp. of water, 16°; air, 16°·75. At 5.30 P.M., lake of Fogliano, temp. of water, 16°·5; air, 15°·5.

There were also placed on the waters of the lakes pieces of perforated cork, glass slides smeared with gelatine or glycerine being placed on the perforations, face downwards, and covered with little roofs of wax-cloth to protect them from the rain.

The examination of No. 9, made at the summer-house of Fogliano, showed some short rods of the length of 2·25—6·75 m. and breadth of 0·225—0·45 m.; one of the length of 11·25 m., and a piece of vegetable epidermis and a cotton fibre.

In No. 11, besides mineral powder and a few vegetable fibres, were found similar rods to those of No. 9, and some long, curved filaments 22·50 micromillimetres in length.

The water taken April 11th in the middle of the lake of Caprolace, and examined three days after, contained a great quantity of motionless bacteria, threads of algæ coloured with chlorophyl, a few infusoria, the following forms of schistomycetes: 1st, delicate filaments, sometimes very long, homogeneous; 2nd, small rounded corpuscles (micrococci ?); 3rd, automatically moving rods of different lengths; 4th, rounded corpuscles arranged in rows; 5th, filaments, partly jointed, containing shining corpuscles. The mud of Caprolace, taken at the same point on the bank as air samples Nos. 10 and 11, contained the same organic forms, a notable quantity of large infusoria, very many diatomaceæ, and some small worms endowed with very active movements, probably larvæ of nematoids.

April 11th, a very small quantity of this mud was introduced by means of a pin into a cultivation tube, filled with very pure fish gelatine, and immediately closed with cotton already kept at a high temp. (Cultivation tube No. 1.)

April 14th, a portion of the Caprolace water was placed in a similar tube and closed by the lamp. (Cultivation tube No. 2.)

Both were placed in a hot-air bath, temp. 30°—34°.

April 15th, a small portion of the contents of cultivation tube No. 1 were placed in a microscopical air chamber. The examination of it showed that the diatoms were all dead; of the nematoids no trace; the filaments of hypomycetes had not undergone further development. Two forms of schistomycetes were seen; some threads, curved, sometimes twisted; others forming loops in which no trace of differentiation of their structure was found, and some rods with a shining granule at each extremity (Table I., fig. 7, *c*); others even smaller contained a third granule in the middle (Table II., fig. 1, *f*). Besides some filaments devoid of nuclei, others exhibited no sign of division (Table II., fig. *a*), whilst in others the protoplasm was in the middle of its length divided into two by a clear space, in which the presence

of a membrane might be discerned (Table II., fig. 1, *b*). Finally, some long and tortuous filaments were like those of fig. 7, which contained a notable quantity of brilliant granules (Table II., fig. 1, *d*). In these last an extraordinarily rapid development was observed, for whilst fig. *d* represented exactly such a filament as seen at midday (April 15), the same filament had at 2 P.M. assumed the appearance of *d'*; that is to say it had increased to almost double the length, had become larger, and contained a quantity of shining corpuscles, which, especially at the fine extremity, had accumulated so as to give to the protoplasm a much more granular appearance than appears in the figure. Two principal forms, therefore, prevail—homogeneous filaments with commencing scission, and others producing spores, in some of which the protoplasm had become granular. It was only by continued observations of the preparation that it was possible to decide that the two forms were connected. A third form (Table II., fig. 1, *e*) consisted of fusiform cells containing very large protoplasmic masses, of which examples are seen in this preparation, belonging to another organic series, and disappearing as the others developed.

The same cultivation examined April 16th, after having been constantly maintained at a temp. 30°—34°, showed a large quantity of very long filaments with many granules, and others with homogeneous protoplasm sometimes arranged parallel to one another, and in some of which were seen manifold transverse divisions (Table II., fig. 3). April 17th, 9 A.M., were found, in addition, very numerous tortuous filaments, especially at the edge of the air channel, the short rods containing two or three nuclei (fig. 7, *c*, and fig. 1, *f*), which the day previous had been observed at points accurately noted, were no longer visible. Instead, the forms were found which are depicted Table II., fig. 2, as shown by the 1-12th inch objective of Zeiss (oil immersion). The smaller figure represents these same shown with eyepiece No. O of Zeiss, and the other two as seen with eyepiece No. IV. They were jointed filaments, each joint of which was limited by a membrane even at the points of division. The smaller of these had a homogeneous protoplasm; the larger contained a granule, very large, oval, shining, and attached to the wall about the middle; or two granules, smaller, and rounded at the ends; or a median and two terminal granules. Some observations have

led us to think it likely that at the place of occurrence of the median granule an ulterior division of joints may take place, but we have not been able to watch the process, which probably is very rapid. The same day at 2 P.M. the articulated filaments with homogeneous protoplasm (fig. 3) were studied with 1-12th inch Zeiss and No. II. eyepiece. The joints were separated from each other by fine interstices, in which could be seen traces of a dividing membrane, rarely clear lines were distinguished between the two extremities of the joint. The joints were disposed in linear series or were dichotomous. We do not know, however, if this dichotomy is the result of dichotomous division of a joint or of lateral deviation of new terminal joints. The latter is much more probable, as we have never been able to see anywhere the beginning of longitudinal division of the protoplasm.

Sometimes the terminal joints are found in a direction perpendicular to that of the preceding, so that one is led to think that they have been detached, and that the position assumed by them is due to resistance accidentally encountered in the fluid in which they are immersed, as well as to the activity of growth of the joints which had pushed them forward.

At this period of evolution all automatic movement of protoplasm is wanting.

April 26th—*i.e.*, after twelve days of cultivation—at a temp. of 30°—34°, were seen along the whole border of the preparation these filaments, in which no further division had been produced.

They were disposed in groups which, leaving the edge of the air chamber, extended in the form of a fan towards the centre of the preparation; one of these, weakly magnified with objective E of Zeiss and eyepiece No. II., is shown in fig. 6, Table II. Although the joints are feebly connected together, the filaments maintain their continuity, run at first parallel, then diverge, and even cross.

The smaller joints have a length of 2·25 m. and breadth of 1·125 m. From these samples one sees how, under the combined influence of the material chosen as the basis of cultivation, of a fixed temperature, and of the action of the atmosphere, many of the organisms contained in the crude material had perished; whilst from the other part had been developed, in great abundance, forms of schistomycetes, belonging to the genus *Bacillus*. The two different species—the one with homogeneous joints, and

the other with joints containing granules or spores—most probably are derived from one another, because sometimes conjoined in the same plant.

This, therefore, would be a *Bacillus*, which might be distinguished from the *B. subtilis* (F. Cohn) of hay infusions, as from the *Bacillus anthracis* (Kach) of carbuncle, and would be described thus:—

"Rods of the length of 5—10 micromillimetres, which, in developing, are converted into tortuous filaments, divided into joints by means of clear spaces in their protoplasm, or, more rarely, by dividing membranes. These filaments, in the surface exposed to the action of air, produce rows of very short joints, and develop in their interior spores before their division into joints, or after this has happened. These spores occupy the middle, or extremities, of the joints, or both at the same time; when the division into joints does not happen they are multiplied by becoming still smaller, and the interior of the filament is filled by a granular mass."

Inasmuch as the same forms are repeatedly produced by like cultivation, practised in fish gelatine, with different kinds of soil, taken from malarious places (whilst other organic forms are not seen except occasionally, or perish, in consequence of the preponderating development of this *Bacillus*), we supposed that perhaps this plant might be the carrier of malaria, which led us to proceed by experiments on animals to test the value of this conjecture.

Our experiments were performed on rabbits of good breed, for the most part very robust, in which autopsies never disclosed any change due to antecedent disease. They were kept in spacious and well-ventilated stalls of wood, placed in a part of the laboratory near our work room, the large windows of which were always kept open. The floor of each was formed of a network of iron wire, and under this a zinc funnel received and conveyed the urine to a vessel outside the stall. The stalls and floor of the room were cleansed most scrupulously. The animals were fed with cabbage leaves, and on their entrance into the laboratory were marked with Roman numerals, which, to avoid confusion, were written with aniline colours on the skin of the back, denuded of fur.

The same number has been used to distinguish the temp.

curve (Tables III., IV., V.) and the "nature print" of the spleen (Table I.) of each animal, to facilitate comparison.

Since, for the most part, parallel researches were made with various substances, we may divide the former into groups.

Group I. *Normal rabbits.*—In order to determine the normal size of the spleen, two very fine rabbits were selected, strong and healthy (XVII. and XVIII.), and killed as soon as they were brought to the laboratory. Death was effected by means of a tight noose round the neck and strong simultaneous traction on the hind legs. This mode was adopted with all our animals except Nos. I. and II., which were killed by bleeding. It procured an immediate death without convulsions, so as to avoid disturbance of the circulation capable of altering the volume of the spleen.

In order to avoid the action of extraneous fluid on the spleen, we abstained from determining the volume of this organ.

Even an exact determination of its weight was difficult, owing to evaporation in an organ which, though small in rabbits, has a relatively very large surface.

We therefore, instead of determining the volume and weight, sought to obtain from the nature-print of the organ, immediately after death, some figures in relation to the weight of the animal's body. We did this by multiplying together the three measurements (L., B., and T.) in millimetres of the print and calculating the value of this parallelopiped, in relation to a chilogramme of the body weight. To this relative value we gave the name *Index of the Spleen*, indicated by the letters I. S.

In these two rabbits all the organs were healthy, and the spleens are represented Table I., Nos. XVII. and XVIII. We obtained the following values:—

Table I.—*Spleen of Normal Rabbits.*

Number.	Weight of Body in Grammes.	Diameters of Spleen in Millimetres.			Product of Three Dimensions.	I. S.
		Length.	Breadth.	Thickness.		
XVII. ...	1,755	38·8	8·2	3·6	1,145	—
XVIII. ..	1,591	38·7	9·4	3·7	1,079	—
Mean ...	1,673	38·75	8·8	3·65	1,112	664

Immediately before death the temperature of XVII. was 39°, and that of XVIII. 38°·6. This temperature, however, is not considered normal, because we often found that the animals on entering the laboratory had a subnormal temperature, and only recovered the normal after 24 hours. The following table shows the means obtained by 7—9 daily measurements, made for nine days on six rabbits.

(Before calculating the means, it was necessary to make the correction of the thermometer, which, as already stated, was 0°·1 C. below the normal.)

TABLE II.—*Calculation of Mean Rectal Temperature of Rabbits.*

Number.	Date of Observation.	Daily Mean.	No. of Daily Measurements.	Place of Observation.
V.	April 30	39·556 C.	9	Rome
,,	May 1	39·516	,,	,,
XII.	,, 9	39·457	7	,,
,,	,, 10	39·483	9	,,
,,	,, 11	39·471	,,	,,
XV.	,, 17	39·162	8	,,
XVI.	,, ,,	39·768	,,	,,
XIX.	June 8	39·368	,,	Prague
XX.	,, ,,	39·475	,,	,,
General mean	= 39°·4745, from 75 measurements.			

We believe we are near the truth in estimating the mean temp. of the rectum in rabbits at 39°·5, and therefore in our temp. curves (Tables III., IV., and V.) we have at this level drawn a well-marked line representing this normal.

In the analysis of pathological cases, we think it convenient to form three groups (II., III., and IV.), according to the source of infecting material used—Pontine marshes, Janiculan, Valchetta in the Roman Campagna. In a fifth group, researches made at Prague in the same way as at Rome; and in the sixth group, cases of septic infection accidentally produced in some animals not used in our experiments, but offering excellent means of comparison with those of malarious infection, artificially induced at the same time and place.

Group II. *Researches with infecting material collected in the Pontine Marshes.*—These, made on Nos. I., II., III., IV., V., served to test the material collected at the lake of Caprolace and in the air of Ninfa and Fogliano, without preparation or after-cultivation (microscopic analysis already given).

A. *Parallel researches with Caprolace mud.*—No. I. was injected April 16th, 10 a.m., with 1·6 cc. of water which had rested three days on Caprolace mud, and immediately afterwards No. II. was injected with 0·6 cc. of fluid from cultivation tube No. I., in which the same mud had been cultivated in fish gelatine since April 11th, and on the same day (April 16th) showed the bacilli provided with spores in fig. 7, *c*, and fig. 1, *f*, Table II.

Table III.—*Temperature and Weights of Nos.* I. *and* II.

Day.	Hour.	Rectal Temp.		Weight of Body in Grammes.		Day.	Hour.	Rectal Temp.		Weight of Body in Grammes.	
		I.	II.	I.	II.			I.	II.	I.	II.
Apr.15	—	—	*	1,560	1,804	Apr.20	11.40 a.m.	40·8	39·8	—	—
„ 16	10 a.m.	39·2	39·0	—	—	„ „	2.30 p.m.	40·5	39·4	—	—
„ „	2.30 p.m.	40·15	39·9	—	—	„ „	3 „	40·5	39·4	—	—
„ „	8 „	39·85	40·2	—	—	„ „	6.40 „	40·7	39·8	—	—
„ 17	9.30 a.m.	39·5	39·7	1,443	1,684	„ „	10 „	40·3	39·6	—	—
„ „	2 p.m.	38·4	38·9	—	—	„ 21	6 a.m.	40·8	39·7	—	—
„ 18	9.40 a.m.	39·4	39·45	1,445	1,708	„ „	8 „	40·7	39·7	†	—
„ „	3 p.m.	38·4	39·7	1,461	1,780	„ „	9.15 „	40·9	—	1,837	—
„ „	5 „	39·75	39·7	—	—	„ „	11.30 „	—	39·65	—	—
„ „	8.30 „	39·7	39·5	—	—	„ „	7 p.m.	—	39·8	—	—
„ 19	9.15 a.m.	40·0	39·6	1,408	1,783	„ „	10.45 „	—	39·8	—	—
„ „	11 „	40·2	39·6	—	—	„ 22	6 a.m.	—	39·8	—	—
„ „	2 p.m.	40·6	40·1	—	—	„ „	12.45 p.m.	—	39·4	—	—
„ „	3 „	40·65	40·05	—	—	„ „	1 „	—	39·9	—	—
„ „	4 „	40·85	40·2	—	—	„ „	3 „	—	39·9	—	—
„ „	5 „	40·8	40·2	—	—	„ 23	12 a.m.	—	39·5	—	—
„ „	6 „	40·5	40·2	—	—	„ „	6 p.m.	—	39·5	—	—
„ „	8 „	40·6	40·6	—	—	„ 24	6 a.m.	—	39·2	—	—
„ „	11 „	40·5	40·3	—	—	„ „	12 „	—	39·6	—	—
„ 20	6 a.m.	40·7	39·6	—	—	„ „	6 p.m.	—	39·8	—	—
„ „	8.10 „	40·6	39·7	—	—	„ 25	7 a.m.	—	39·5	—	—
„ „	8.10 „	40·65	39·8	—	—						

* Subcutaneous injection of above mentioned material into both rabbits.
† No. 1 killed by bleeding.

Add correction 0°·1 in each table.

Autopsy of No. I., immediately after death. At injection site large callosity (4 cm. × 3·5 cm.) of whitish and apparently fibrous connective tissue, containing blood vessels filled with

hard, dark red thrombi, subcutaneous tissue slightly œdematous. The œdema fluid, collected immediately by means of capillary tubes, contains a great quantity of automatically moving corpuscles, of oval or rounded form, shining, some showing on opposite sides small blunt prolongations. In addition, immovable or feebly moving filaments, 5·9001 m. max. L.: 0·7143 m. B. Mesenteric lymphatic glands swollen, and contain large quantity of opaline fluid. Spleen, enlarged: 55 mm. L., 9 B., and 4 T. Examination of its fresh tissue shows a great quantity of dark brown pigment in irregular masses; adding aqueous humour taken from eye of the same rabbit, and perfectly normal, a great number of actively moving rounded corpuscles were seen. No change in other organs.

To determine well the nature of the rounded bodies in the splenic pulp and lymphatic fluid, two air cells were employed (Nos. 7 and 8). In the first was placed serum of the lymph, in the second a little splenic pulp diluted with aqueous humour. Both were maintained at 30°—35° (the glasses having previously been heated to 120° to kill any organisms on them). After 24 hours No. 7 contained motionless filaments with brilliant granules (spores) and self-moving rods, sometimes united in couples, besides free oval bodies, like those in the filaments (Table II., fig. 4). In No. 8 were formed, besides many free spores, some filaments with homogeneous protoplasm.

These, especially the smaller ones, could only be seen with 1-12 or 1-18 inch objective of Zeiss and Abbé illumination. (Afterwards they were seen well with weaker objectives.)

In both rabbits a fever of intermittent character followed the injection of the above substances (Table III., curves I. and II.). After injection an immediate rise to 40° took place; next day, a fall below the normal; 3rd day, a slight rise; 4th, a new F. A. (febrile *accès*) to 40·85 in No. I., and to 40·6 in No. II. After the 5th day the course was different in the two. No. I. maintained a high temp. for 48 hours with slight oscillations, whilst No. II. showed slight rises on the 5th, 6th, and 7th days, with daily remissions; on the 8th, a normal temp.; on the 9th, a subnormal in the morning and a slight rise in the afternoon. On the 10th, used for another experiment.

We see, therefore, the fever in No. I. continue after the second F. A., and in No. II. an almost normal condition after the second

F. A.—a difference dependent upon the quantity of infecting material used.

As to the *post-mortem* appearances met with in No. I.—killed during the F. A.—we note, 1st, the absence of suppuration at injection site; 2nd, the increase, in all dimensions, of the spleen, especially its length, I. S. 1,298, *i.e.*, almost double the normal (664); 3rd, the presence of the organisms of the injected liquid, in the injection site, spleen, and lymph. Neither the course nor *post-mortem* conditions correspond to those of putrid and septic fevers. Also the increase of weight between first and second F. A.

B. *Parallel researches with Caprolace mud and with material collected in the air of Ninfa and Fogliano,* to ascertain if the infecting agent is, even before the fevers appear, diffused in the lower strata of the air of such places. It is not unlikely that the greater activity displayed by the sample from Fogliano, where the odour of putrescence was strong, may point, in accordance with the ideas of the inhabitants, to a greater infective power.

April 17th were begun some cultivations with the samples of air Nos. 5 and 7, collected at Ninfa and Lake Fogliano. The isinglass with which the slides had been smeared was dried: a portion placed with albumen in two air cells (microscopic), a second in two tubes of very pure fish jelly, and a third in urine. The latter had long been boiled in a small retort, which was afterwards closed with cotton, and remained perfectly clear during 24 hours. The cultivation with albumen gave no result. In the tubes of fish gelatine the development of bacteria took place, and was reserved for other researches. The urine cultivation was used at once. It was pale yellow, clear, and acid; contained long motionless filaments, partly jointed, and in some of the joints spores were seen.

April 25th, 1·6 cc. of this urine were injected under the skin of No. II., and simultaneously No. III. (not used before in any experiment) was injected with 3 cc. of water, which had long stood on Caprolace mud, containing some oval corpuscles and slender, twisted filaments.* There were also injected into another rabbit, No. IV., 1·6 cc. of a cultivation tube in which

* The microscopic examinations were always made by both observers, and separately recorded by each.

had been placed, April 23rd, a very small quantity of Caprolace mud with fish gelatine. In this liquid were seen only small shining granules.

The results are seen in the following table :—

TABLE IV.—*Temperature and Weights of Nos.* II., III., *and* IV.

Day.	Hour.	Temperature.			Weight of Body.			Observations.
		II.	III.	IV.	II.	III.	IV.	
Apr. 25	7 a.m.	39·5	—	—				
" "	8 "	—	38·9	38·5				
" "	9 "	—	—	—	1,686	1,546	1,824	Injection.
" "	10 "	39·4	39·3	39·1				
" "	12 "	39·6	39·6	39·2				
" "	2 p.m.	39·6	39·2	38·8				
" "	4 "	39·7	39·3	38·7				
" "	6 "	39·9	39·2	39·0				
" "	8 "	39·8	39·2	39·2				
" "	10 "	39·8	39·2	39·2				
" 26	6 a.m.	39·4	38·9	39·1				
" "	8 "	39·6	39·4*a*	38·75	*b* —	—	—	*a.* During the movements of the animal, 39·6.
" "	10.15 "	39·4	39·4	39·8				*b.* At 8.30 a.m. No. IV. injected with 3·4 cc. of the same cultivation.
" "	12 "	39·35	39·5	38·5				
" "	2 p.m.	39·5	39·0	38·6				
" "	4 "	39·6	39·3	39·0				
" "	6 "	39·7	39·5	39·5	—	—	—	No. IV. had six young ones.
" "	8 "	39·5	39·4	39·4				
" "	10 "	39·6	39·4	39·4				
" 27	6 a.m.	39·3	39·2	39·5				
" "	8 "	39·6	39·3	39·45	1,717	1,871	1,532	The weight of the young ones was 262; that of the mother, No. IV., 1,532: total, 1,794.
" "	10 "	39·4*c*	39·2*d*	39·6	—	—	—	*c.* Injection of 3 cc. of cultivation in urine of sample of air of Ninfa and Fogliano.
" "	12 "	39·5	40·5	39·65				*d.* 3 cc. of water from mud of Caprolace injected.
" "	2 p.m.	39·7	40·1	40·05				
" "	4 "	39·7	40·2	39·9				
" "	6 "	39·8	40·2	39·6				
" "	8 "	39·8	39·9	39·8				
" "	10 "	39·8	39·9	39·9	1,833	1,615	1,530	
" 28	6 a.m.	39·65	39·3	39·7				
" "	8 "	39·7	39·4	39·6				
" "	10 "	39·3	39·25	39·5				
" "	12 "	39·4	39·3	39·4				
" "	2 p.m.	39·5	39·45	39·5				
" "	4 "	39·75	39·4	39·45				
" "	6 "	39·6	39·4	39·7				
" "	8 "	39·6	39·5	39·65				
" "	10 "	39·65	39·25	39·8				
" 29	6 a.m.	39·4	39·3	40·05				
" "	8 "	39·6	39·0	41·00				
" "	10 "	39·5	39·2	39·9				
" "	12 "	39·6	39·2	40·2				
" "	2 p.m.	39·4	39·3	40·05				
" "	6 "	39·6	39·2	40·05				
" "	8 "	39·95	39·55	40·3				
" "	10 "	39·8	39·45	41·05				
" 30	6 a.m.	41·0	39·1	41·0				
" "	8 "	39·95	39·2	39·8				
" "	10 "	40·2	39·0	40·2	1,749	1,668	1,632	
" "	12 "	40·1	39·2	40·4				

Temperatures and Weights of Nos. II., III., *and* IV.—*Continued.*

Day.	Hour.	Temperature.			Weight of Body.			Observations.
		II.	III.	IV.	II	III.	IV.	
Apr. 30	2 p.m.	39·8	39·2	40 2				
,, ,,	4 ,,	39·5	39·2	40·2				
,, ,,	6 ,,	39·55	39·3	40·15				
,, ,,	8 ,,	39·4	39·2	39·9				
,, ,,	10 ,,	39·5	39·2	40·1				
May 1	6 a.m.	39·5	39·2	40·1				
,, ,,	8 ,,	39·35	39·4	39·9				
,, ,,	10 ,,	39·1	39·5	39·85				
,, ,,	12 ,,	39·25	39·3	39·95				
,, ,,	2 p.m.	39·45	39·2	40·05				
,, ,,	4 ,,	39·4	39·3	39·9				
,, ,,	6 ,,	39·4	39·1	40·0				
,, ,,	8 ,,	39·5	39·4	40·1				
,, ,,	10 ,,	39·6	39·3	40·0				
,, 2	6 a.m.	39·3	39·4	40·1				
,, ,,	8 ,,	39·4	39·1	39·85				
,, ,,	10 ,,	39·2	39·3	39·9				
,, ,,	12 ,,	39·5	39·2	39·6				
,, ,,	2 p.m.	39·5	39·2	39·7	1,745	1,612	1,490	
,, ,,	4 ,,	39·4	39·3*e*	39·9	—	—	—	*e.* At 3.30 p.m. No. III. injected with 16 cc. of filtered fluid of cultivation of Ninfa and Fogliano air, in urine. See parallel experiment following.
,, ,,	6 ,,	39·7	—	40·5				
,, ,,	8 ,,	39·8	—	39·9				
,, ,,	10 ,,	40·0	—	39·95				
,, 3	6 a.m.	39·5	—	*f*	—	—	—	*f.* Thermometer broken. New one also. 0·1 below normal.
,, ,,	12 ,,	39·2	—	—				
,, ,,	2 p.m.	39·85	—	40 0				
,, ,,	4 ,,	39·7	—	40·0				
,, ,,	6 ,,	40 0	—	40·1				
,, ,,	8 ,,	40·1	—	39·8				
,, ,,	10 ,,	40·0	—	39·9				
,, 4	6 a.m.	39·9	—	40·1				
,, ,,	8 ,,	39·5	—	39·9				
,, ,,	12 ,,	39·6	—	40·0				
,, ,,	2 p.m.	40·0	—	40·1				
,, ,,	4 ,,	40·1	—	40 0				
,, ,,	6 ,,	39·8	—	40 05				
,, ,,	8 ,,	39·85	—	40·0				
,, ,,	10 ,,	39·7	—	40·0				
,, 5	6 a.m.	39·5	—	40·0				
,, ,,	8 ,,	39·7	—	39·8				
,, ,,	12 ,,	39·7	—	39·2	1,910	—	1,529	
,, ,,	2 p.m.	39·8	—	36·6	—	—	—	At 2.30 No. IV. killed.
,, ,,	4 ,,	39·8	—	—				
,, ,,	6 ,,	40·0	—	—				
,, ,,	8 ,,	40·1	—	—				
,, ,,	10 ,,	40·0	—	—				

Temp. table of No. III. will be continued below.

No. II. killed May 6th by bleeding. At 6 A.M. of this day its rectal temperature was 39°·9; at 8 A.M., 39°·8; at 10 A.M. 39°·4. Weight, 1,602 grammes.

Autopsy.—Well nourished (on the 11th day of observation it weighed 224 gr., and on the day of death 116 gr., more than

when it came to the laboratory). Spleen much enlarged (Table I., fig. 2.); L. 52·6 mm.; B. 11·5 mm.; T. 5·8 mm.; I. S. (compared with maximum weight on 11th day) 1,832, *i.e.*, almost treble the normal—firm, not rich in blood, of clear red colour, and its capsule wrinkled. Intestinal glands not swollen, but mesenteric lymphatics much enlarged, forming at the root of mesentery a mass—3 cm. × 1·8 cm.—firm, whitish, and containing much lymph. Liver rich in blood. Kidneys less so. Slight pulmonary œdema, with subpleural ecchymoses. At one injection site a small capsuled abscess.

Temp. curve (Table III., curve II.) very interesting. First two days after first injection temp. oscillations slight. First 48 hours after second injection notable perturbations observed. Temp. commenced to rise rapidly 51 hours after second injection, arriving in 16 hours at 41°. The F. A. had a duration of 24 hours.

In 36 hours following, minima of 39°·1—39°·2, and maxima almost normal. Then a series of 4 F. A. (*f*, *g*, *h*, *i*), each lasting about 24 hours, with maxima of 40°, 40°·1, 40°·1, 40°·1, and maxima of 39°·2, 39°·5, 39°·5.

The quartan type of fever after F. A.—f. was transformed into a quotidian type.

The *post-mortem* appearances exclude septic complication.

Melanæmic pigment was found in the spleen. In the lymph were found many brilliant actively moving oval bodies, 0·00095 mm. long.

It results, therefore, that the air examined contained corpuscles capable of development, which, after multiplication in urine, were rendered capable of producing attacks of intermittent fever in the animals injected with them.

To get this result it was necessary to use a considerable quantity of cultivation liquid, and it occurred after a period of latency of 48 hours. This is explained by the large quantity of fluid (400 cc.) in which was diffused the material in cultivation.

It remained to be seen if the febrigenous action was due to the solid particles in this liquid, or to some other substance in it. And to this end we made the following experiment:—

No. III., already injected twice with water from Caprolace mud (1·6 cc. April 25th, 3·2 cc. April 27th), showed two slight rises after the first injection (Table III., curve III., *a*, *b*), the

maxima being 24 hours apart, and the difference of max. and min. only 0°·7.

All this curve is subnormal. After the second min. the temp. ascended to the mean. (Such oscillations are very often seen in rabbits placed under new conditions of life, food, &c., and have nothing to do with intermittent fever. Of this in the present case we had a positive proof.) When the temp. had become normal, a double dose of the same fluid was injected, and the temp. at once rose to 40°·2, then fell, so that for five days the curve was subnormal, only once passing the normal by 0°·1. Therefore the two injections were powerless to produce an intermittent fever, or in other words, *water which had long been in contact with mud rich in malaria did not seem apt to transmit this poison.*

The negative result in this case was not due to immunity of the animal, because a later experiment on it showed that such immunity did not exist. This result has an important bearing on the fact, that the natural development of malaria is known to be wanting when a sufficient layer of water covers and separates the soil from the atmosphere. The Caprolace water was in our researches shown to be devoid of those organisms which may be developed in such quantities from cultivations made with the mud itself, which produced such positive results in No. II.

This water was rich in self-moving schistomycetes, but in no cultivation with fish gelatine, albumen, urine, or even left to itself in flasks closed with cotton, were bacilli ever seen to develop containing nuclei. The forms were always such as one of us (Klebs) has described as *monadine*, which goes to prove that the bacillus described by us is eminently *ærobic*, which only grows in contact with the air, and accords with the conditions of malaria development set forth in Chapter I., and with our researches, in which the plant always grew on the surface of the fluid.

No. IV., the third of this series, which was injected with 1·6 cc. of a cultivation of the bacillus in fish gelatine, was in conformation and origin like No. III. Both were strong albinoes, probably of one litter, and were kept in the same stall.

No. IV. had also at first a subnormal temp. In the first days after injection it showed slight temp. oscillations, but always subnormal. Towards the middle of the third day, temp. rose

above the mean, and at 2 P.M. surpassed 40°, then slowly fell to 39°·4 on the day following. On the fifth there was a strong F. A. (max. 41°), *so that, as happens in the tertian type, the two accesses were separated by a day of complete apyrexia.* But the same evening there was a stronger and more lasting rise of eight hours' duration, temp. 41°·05. For five days the temp. remained high (40°) with slight exacerbations and remissions. *So that after the strong accès of eight hours the fever became subcontinued.*

On the sixth day the fever declined (temp. 39°·2). The animal was killed. Before entering on the details of the autopsy, we think it would be useful to note a fact which seems important. Our expectations of positive results in Nos. III. and IV. were at first illusory, and the sudden strong *accès* of the 29th took us by surprise. It is noteworthy that on this day heavy falls of rain occurred, and a great fall of temp., and that in No. II., on which three days before had been practised the last injection, there took place a notable rise immediately succeeding the strong *accès*, *e* (Table III., curves I., II.) On the contrary, Nos. V. and VI., not yet used for experiments, and No. III., which had been injected with an inactive material, showed a *conspicuous fall* of temp.

It seems that the infected animals resisted this atmospheric change differently to the non-infected ones.

The following data, furnished by the Director of the Observatory of the Roman College, show what these were :—

Day.	Barometer Reduced to Sea Level.	Temp.		Humidity per cent.	Rain in Millimetres.	Wind.
		Max.	Min.			
April 25	756·6	19·7	9·1	66	3·3	N., S.W.
,, 26	759·5	19·1	7·0	68	drops	N., S.W.
,, 27	758·7	19·2	12·1	69	2·7	S.W., S.E., N.W.
,, 28	754·5	16·7	9·7	66	11·3	E., S.E., S.W.
,, 29	754·0	13·2	9·4	88	66·0	N., N.E.
,, 30	756·8	15·0	8·3	79	8·0	N., N.W.
May 1	759·0	17·9	8·3	68	0	N., S.W.
,, 2	758·8	15·3	7·5	78	6·0	S.E.
,, 3	755·3	14·5	8·2	80	30·2	S.E.
,, 4	757·8	16·5	8·0	71	10·0	N., S.W.
,, 5	760·9	18·0	8·0	83	22·4	N., S.W., N.

We see corresponding to the 29th, lower temp.; diminished atmospheric pressure; greater humidity; heavy rain. On the

30th the atmospheric pressure increases, but the min. temp. is still lower.

It is therefore probable that cooling of the air and increase of its humidity had influenced the production of the strong febrile accesses in the infected, and had determined a fall of temp. in the non-infected animals.

Autopsy, No. IV.—Well nourished; had gained W. 63 gr. since April 26th. Spleen greatly enlarged (Table I., fig. 4); L. 67·3 mm.; B. 13 mm.; T. 4·3 mm.; I. S. 2,460, *i.e.*, four times the normal, contracted, capsule wrinkled, and poor in blood. Liver and kidneys congested, but normal. Mesenteric glands enlarged, whitish, rich in lymph. Patches of Peyer slightly elevated, and greyish white. Intestinal mucous membrane slightly reddened. Uterus large, placental insertion faint, mucous membrane smooth, reddish. Lungs large, subpleural ecchymoses. Heart contracted. Abdominal veins contain much fluid blood. In the lymph many oval shining corpuscles with active movement; a few also in the spleen. Cultivations were made with blood collected in capillary tubes. In one bacillus development took place. In the spleen were many large cells containing fragments of red globules and many shining granules; much brown and a considerable quantity of black pigment in this spleen, which pigment was stained blue by ferrocyanide of potassium and hydrochloric acid.

C. *Parallel experiments on the efficacy of fluid obtained by cultivation in urine of samples of air collected in Ninfa and Fogliano.*—These had for their object to discover if any difference existed between the action of the cultivation fluid before and after filtration.

The cultivation fluid, by means of which the malarious infection had already been produced in No. II., was filtered through a cell of plaster of Paris by means of a Bunsen's pump.

Sixteen cc. of filtered fluid were injected, May 2nd, by several punctures under the skin of the back of No. III., which had since April 29th onwards had a normal temperature, and from previous researches might be supposed to have a predisposition to resist malarious infection.

Into No. V., not used in any previous experiment, was injected only 3·2 cc. of the liquid remaining in the filter.

TABLE V.—*Temperatures and Weights of Nos. III. and V.*

Day.	Hour.	Temperatures and Weights. III.	Temperatures and Weights. V.	Difference in Temperature of No. V. from III.	Day.	Hour.	Temperatures and Weights. III.	Temperatures and Weights. V.	Difference in Temperature of No. V. from III.
May 2	6 a.m.	39·1	39·7	+ 0·3	May 6	8 p.m.	39·8	40·1	+ 0·3
" "	8 "	39·1	39·6	+ 0·5	" "	10 "	39 5	40·8	+ 0·5
" "	10 "	39·3	39·5	+ 0·2	" 7	6 a.m.	39·55	39·8	+ 0·25
" "	12 "	39·2	39·6	+ 0·4	" "	8 "	39·7	39·9	+ 0·2
" "	2 p.m.	39·2	39·5	+ 0·3	" "	10 "	39·5	39·9	+ 0·4
" "	—	1612gr.	1798gr.	—	" "	12 noon	39·3	39·8	+ 0·5
" "	4 "	39·3	39·4	+ 0·1*	" "	2 p.m.	39·4	40·1	+ 0·7
" "	6 "	39·8	40·0	+ 0·2	" "	4 "	39·7	40·1	+ 0·4
" "	8 "	40·1	40·42	+ 0·32	" "	6 "	40·0	40·2	+ 0·2
" "	10 "	40·25	40·2	— 0·05	" "	8 "	39·7	40·1	+ 0·4
" "	12 "	40·2	39·9	— 0·03	" "	10 "	39·8	40·0	+ 0·2
" 3	12 noon	39·1	40·4	+ 1·3	" 8	6 a.m.	39·3	40·0	+ 0·7
" "	2 p.m.	39·5	40·2	+ 0·7	" "	8 "	39·65	39·9	+ 0·25
" "	4 "	39·35	40 2	+ 0·85	" "	10 "	39·3	39·85	+ 0·55
" "	6 "	39·7	40·3	+ 0·6	" "	12 "	39·4	39·9	+ 0·5
" "	8 "	39·8	40·3	+ 0·5	" "	2 p.m.	39·2	40·0	+ 0·8
" "	10 "	39·6	40·1	+ 0·5	" "	4 "	39·5	40·1	+ 0·55
" 4	6 a.m.	39·5	40·0	+ 0·5	" "	6 "	39·6	40·3	+ 0·7
" "	8 "	39·65	39·85	+ 0·2	" "	8 "	39 5	40·0	+ 0·5
" "	12 noon	39·7	39·6	— 0·1	" "	10 "	39·4	39 9	+ 0·5
" "	2 p.m.	39·8	39·5	— 0·2	" 9	6 a.m.	39·4	39·8	+ 0·4
" "	4 "	39·5	40·0	+ 0·5	" "	8 "	39·2	39·7	+ 0·5
" "	6 "	39·4	40·0	+ 0·6	" "	10 "	39·2	39·9	+ 0·7
" "	8 "	39·6	40·05	+ 0·45	" "	2 p.m.	39·5	40·0	+ 0·5
" "	10 "	39·55	39·95	+ 0·4	" "	4 "	—	40·0	
" 5	6 a.m.	39·2	39·9	+ 0·7	" "	6 "	39·7	39·9	+ 0·2
" "	8 "	40·0	39·85	— 0·15	" "	8 "	39·6	39·95	+ 0·35
" "	11 "	1657gr.	1767gr	—	" "	10 "	39·65	40·0	+ 0·35
" "	12 noon	39·35	39·9	+ 0·55	" 10	6 a.m.	39·3	39·9	+ 0·6
" "	2 p.m.	39·7	39·8	+ 0·1	" "	8 "	39·6	39·9	+ 0·3
" "	4 "	39·6	40·0	+ 0·4	" "	10 "	39·6	39·85	+ 0·25
" "	6 "	39·7	40·5	+ 0·8	" "	12 "	39·5	39·9	+ 0·4
" "	8 "	39·8	40·4	+ 0 6	" "	2 p.m.	39·4	40·0	+ 0·6
" "	10 "	39·7	40·1	+ 0·4	" "	4 "	39·6	40·05	+ 0·45
" 6	6 a.m.	39·3	39·6	+ 0·3	" "	6 "	39·7	40·0	+ 0·3
" "	8 "	39·7	39·8	+ 0·1	" "	8 "	39·8	39·9	+ 0·1
" "	10 "	39·1	39·7	+ 0·6	" "	10 "	39·6	39·85	+ 0·25
" "	12 noon	39·3	39·9	+ 0·6	" 11	6 a.m.	39·5	39·9	+ 0·4
" "	2 p.m.	39·4	40·0	+ 0·6	" "	8 "	39·4	39·7	+ 0·3
" "	4 "	39·6	40·15	+ 0·55	" "	10 "	1759gr	1807gr.	†
" "	6 "	39·7	40·1	+ 0·4					

* Injection of both. † Both killed.

Autopsy.—Results identical with the cases explained further back. No suppuration at injection sites, not even No. III., in which there were so many punctures; sup. mesenteric glands enlarged. Both spleens enlarged, but in different proportions. (Table I., figs. 3 and 5.)

	L.	B.	T.	W. of Body.	I. S.
No. III.	51 mm. ...	11·8 ...	4·2 ...	1,757 ...	1,410
" V.	56 " ...	11·2 ...	5·6 ...	1,807 ...	1,943

Whilst spleen No. III. contained no pigment except in the form of small granules of a rusty colour, enclosed in a few white cells scattered widely apart, that of No. V. was rich in pigment. The most of this was nickel-coloured, and enclosed in the white cells as granules or in very voluminous masses, or had taken the place of hæmoglobin in the red globules. Some of these had preserved their dimensions, homogeneity, and discoid shape, but the hæmoglobin was replaced by pigment. Besides the nickel-coloured, a certain amount of black pigment was met with in the spleen, chiefly as granules enclosed in white cells. These results and the temp. curves (Table III., curves III. and V.) show that a malarious infection was produced in both, but of less intensity by the filtered than by the unfiltered fluid, although No. III. was weakened by a previous experiment.

The fever type in V. was quotidian, with max. intensity at the 1st, 4th, and 7th F. A., as if it was a combination of a quotidian and a tertian. Without wishing to assign an undue value to the manifold distinctions in the simple and compound forms of intermittent, we think it very probable that the great variety of type depends on corresponding variety in the intensity of the infecting agent, which may have a diagnostic importance. Probably very frequent measurements of the temp. in man would tend to increase the number of recognized forms.

This experiment tells in favour of the conclusion that separation of the solid particles of an infecting fluid robs it of much of its power and generates a mild type of fever. The degree of completeness of the separation is, in our cases, uncertain, but at all events it goes to confirm what was discovered by cultivations, viz., *that the solid particles capable of development are the carriers of the virus;* however, we cannot yet absolutely exclude the possibility that the fluid in which the organisms are suspended, and in which the products of their material change are found, may concur in the morbigenous action they undoubtedly possess. Experiments on Nos. XV. and XVI., and on VIII. and XII., gave an analogous result.

D. *Parallel researches with the bacillus malariæ cultivated in an open and a closed vessel.*

April 23rd.—To two cultivation tubes of fish gelatine was added a very small quantity of Caprolace mud, one sealed by the

lamp, the other plugged with cotton, previously kept at a high temp.; both maintained at 30° to 50°.

May 5th.—A whitish scum appeared on the first tube denser than that on the second. Examined microscopically, motionless rods were seen in the first cultivation, and others which oscillated gently. In the first were brilliant nuclei, which were wanting in the second; in addition many free oval spores were seen. In the second cultivation only rods and motionless filaments with spores. Tubes closed, and, May 9th, contents used for two parallel researches.

May 9th, 10 A.M.—No. IX. injected with 1·04 cc. of open cultivation, and No. X. with 3·2 cc. of closed cultivation.

No. IX. had from the commencement a strong F. A. of 24 hours' duration, with two max. temp. of 40°·3 and 40°·2, and interposed min. of 39°·45 (Table IV., curve IX.). On the 11th, 12th, and 13th the temp. was normal, with rather marked oscillations; 14th appeared a F. A., which lasted 48 hours, and in which three notable rises of temp. were observed with intervening falls, during which the temp. was always above the normal (curve IX., *a*, *b*, *c*). May 16th, 6 A.M., temperature still 40°; at 10 A.M. fell to 39°·2, *i.e.*, sub-normal; 17th, 18th, and 19th, temp. at or below normal; 20th, in the morning aborted [two]; temp. at 6 P.M., 39°·8. Killed.

No. X. Very similar temp. curve (Table IV., c. X.); first F. A. after injection and two maxima, but nearer together. Three days following temp. fell, but always above the normal. 13th, 6 A.M., temp. 40°·05. This second *accès* commenced a day earlier than in No. IX., lasted three days, and showed four chief phases of recrudescence and remission (c. X., *a*, b^2, *c*, *d*). The maxima and minima are a little lower than in No. IX. On five succeeding days temp. above, and then fell to normal.

Although in No. X. the quantity of injected material was greater than in No. IX., the first F. A. was shorter, and the second represented a subcontinuous of less intensity and longer duration. It is probable the infective power of the closed was less than that of the open cultivation, and would have been more marked if the former had not contained a notable quantity of air. In fact, in the cultivations made with absolute exclusion of air, the development of the bacillus was suspended, and they could not be used for experiment.

The following table shows that after the injection and first F. A. they gained weight, and this in No. X. continued up to death:—

	IX.		X.	
May 8	1,902 gr.	......	1,819 gr.	
,, 9	1,818 ,,	......	1,715 ,,	Injection.
,, 16	1,898 ,,	......	1,796 ,,	After second *accès* of fever.
,, 17	1,775 ,,	......	1,818 ,,	
,, 18	1,777 ,,	......	—	
,, 20	—	......	—	No. IX. aborted (weight of two abortions, 65gr.)
,, 21	1,596 ,,	......	1,847 ,,	

Both killed May 21st. No. IX., spleen moderately enlarged, firm and dark-coloured; that of No. X. more voluminous, clearer coloured, and softish. In both slight pulmonary œdema, and in No. X. intra-alveolar extravasations. Size of spleen as follows (Table I., figs. 9 and 10):—

	L.	B.	T.	I. S.
No. IX.	46·0 mm.	10·8 mm.	3·0 mm.	934
,, X.	60·8 ,,	13·7 ,,	6·1 ,,	2,754

These two experiments prove that bacilli cultivated in fish gelatine produce intermittent fevers, but do not establish a decided difference between those cultivated in open and in closed vessels.

Group III. *Researches with samples of soil of the Janiculan.*—Dr. Fleischl, physician in Rome, politely informed us that Signor W., proprietor of the Villa Spada, on the Janiculan, had been attacked by a grave form of fever, at a time when such had not yet appeared in Rome. This was owing to a great disturbance of the soil near the villa, in immediate proximity to the bedroom of Signor W. (on the ground floor). These excavations had been made in soil rich in *humus*, which had long been used as a garden, and in a clayey soil lower down, where an orange plantation was being made. May 2nd we brought to the laboratory specimens of both kinds of soil, taken from below the superficial layers of soil, and made with them artificial marshes, as described in Chapter II., which were kept in an air bath at a temp. of 35°—36°. In water mixed with these soils were seen

very many self-moving oval corpuscles, of max. diam. 0·00095 mm., sometimes united in twos and threes, in rows which oscillated. In specimens of soil from the new Botanic Gardens on the Viminal, used for comparison, these bodies were found but sparingly, and never united in rows.

May 6th.—Nos. VII. and VIII., not previously employed for experiment, were injected with mixtures of these soils, from the artificial lakes, with water—No. VII., 6·4 cc. of fluid from clayey soil, and No. VIII., the same quantity from soil rich in *humus*.

No. VII. had in eight days four F. A., with ever-increasing temp. (1st, 39°·9; 2nd, 39°·8; 3rd, 40°·3; 4th, 40°·3, 40°.1, 40°·3). Even in the remissions the temp. went on increasing. In the next six days the fever assumed the quotidian type, and the max. rose gradually to 41°·1.

No. VIII. from May 6th to 16th showed much less marked temp. rises. The maxima oscillated between 39°·9 and 40°·05; the two first 24 hours, and others 48 hours apart.

TABLE OF WEIGHTS.

	VII.	VIII.	Observations.
May 6	... 2,129 gr.	... 1,838 gr.	Injections practised.
,, 14	... —	... —	No. VII. had seven young ones, not weighed.
,, 16	... 2,092 ,,	... 1,898 ,,	
,, 17	... —	... 1,878 ,,	
,, 19	... —	... —	No. VIII. had five young ones, weighing 214 gr.
,, 20	... 1,965 ,,	... 1,719 ,,	
		+ 214 ,,	
		1,933 gr.	

May 20th, both killed. *Autopsy*, No. VII.—Spleen much enlarged (L. 56·3; B. 12·8; T. 3·5 mm.; I. S. 1,289), colour darker and consistence less than normal; small quantity of black pigment. Many ecchymoses of pleura, and in superior (in man, posterior) parts of lungs diffused hyperæmia and œdema. Right heart gorged with fluid blood. Injection sites normal.

No. VIII., spleen less enlarged (L. 49·0; B. 11·0; T. 3·0; I. S. 837). Normal colour and consistence; very few granules

of black, and considerable quantity of dark red pigment; sanguinolent fluid in pleural cavities; partial atelectasis of lungs with slight emphysema of lower (in man, anterior) border. Injection sites normal.

The results of these experiments point to the great difference of development of the agent in virgin soils, compared with those long under garden culture. It would seem as if the latter reduced the danger to a minimum, but one experiment with soils from one place is not conclusive.

Group IV. *Experiments with samples of soils of Agro-Romano.*—The soils used in these were taken from the farm of Valchetta (Cav. Francesco Piacentini). The first from a marsh situated on one of the hills. The second from a marsh near the hamlet. The third from a pasture lately broken up by the plough in the valley of the Cremera. All collected May 9th. A fourth from an artichoke ground was received May 17th from Signor A. Piacentini.

With the first three artificial marshes were farmed and kept at 30°—35°. May 14th a first experiment was performed with earth from the marsh near the hamlet. A portion was mixed with an equal volume of Marcia water,* and after the heavier particles had subsided 6·4 cc. were injected under the dorsal integument of No. XII., which had had a slightly subnormal temp. for six days, except on the 13th (Table V., curve XII.). After the injection the temp. rose to 38°·6, and then fell below the normal. 15th, about noon, it rose to 39°·9, and again descended below the normal, 38°·6. These brief and slight rises of temp. were not considered to be due to the injection, as a similar one had occurred the day before it was practised. It was used therefore in another experiment.

With soil from the hill marsh two parallel experiments were instituted, the injection fluid being prepared as before, and No. XII. was injected (May 16th) with 6·4 cc. It was then filtered through Swedish paper, and 6·4 cc. of the filtered liquid injected into No. VIII., already used in an experiment (see last group). After injection the temp. in No. XIII. rose to 40°·2, then fell below the normal. On the 17th, at 10 A.M., a second injection was used of an equal quantity of the filtered fluid.

* A very pure Roman water.

The temp. rose for a short time to 40°, but was not renewed. Animal killed May 20th. The filtered fluid had produced an intermittent fever, with slightly elevated maxima. In last intermission temp. subnormal.

No. XII., which had been simultaneously injected with an equal quantity of the same liquid *unfiltered*, had in 36 hours 3 F. A., with always higher max. and min. Two maxima reached 40°·8 and 40°·6, then rapidly diminished, and the last temp. registered (10 P.M.) was 39°·4. The night of 17th the animal died.

From 16th to 17th it had lost 166 gr. On the 16th it had one abortion, weighing, 30 gr. *Autopsy.*—Blood coagulated—a second abortion protruding from vaginal orifice. Suppuration at injection sites.

The usual oval self-moving corpuscles mixed with the pus cells in large quantity. Many filaments of *bacillus malaria*, some very long. Some of the joints contained spores.

Spleen much enlarged (Table I., fig. 12), it had reached dimensions never before witnessed by us in these malarious infections; L. 81·8 mm.; B. 19·4; T. 6·9; I. S. = 5,903, *i.e.*, almost eight times the normal. It was rather soft, but its angles and borders were not rounded; *the transverse section was triangular, the colour slate. In the splenic pulp examined fresh, were seen many oval self-moving spores, some bacilli. L.* 0·001—0·002, *and even long or homogeneous filaments. Many cells of the spleen contain granules of black pigment. Even the bone marrow contained many self-moving spores and bacilli. In addition were found long and homogeneous filaments of the width of* 0·0006 *mm., and mean length of* 0·06 *mm., some reached a length of* 0·084 *mm.* All these observations were made with a solution of 0·75 per cent. of chloride of sodium in distilled water, freshly prepared, and in which a most careful examination failed to detect any trace of organisms. Without adding any liquid, however, we could observe these filaments in the bone marrow and the spleen. Professor Bizzozero, who was in Rome at the time, kindly assisted in these observations and measurements. The kidneys were very large, especially the left. Their upper surface was bounded by the limit of the inflammatory foci in the injection sites. The liver was dark-coloured and very large. The uterus contained four immature young ones. The right heart

was gorged with grumous blood. Both lungs œdematous with diffuse hyperæmia, and some recent ecchymoses. We here see rise up from the action of the liquid prepared from the hill marsh (which after filtering produced the weak infection in No. VIII.) a true pernicious fever, which killed the animal in 36 hours, and in the course of which, *bacillus malariæ*, diffused through the body, accumulated in the spleen and bone marrow, where it attained a very high development.* The doubts which might in this case exist, from the complication of suppurations in the back, will vanish from the study of the following case, in which the malarious infection produced by the same material ran its course without complication of any kind. This complication was probably due to the fact that the earth employed contained much excrementitious matter, and might easily cause suppurative inflammation. Of a liquid prepared in the same way from earth derived from the hill marsh of Valchetta unfiltered, were injected 6·4 cc. into the back of No. XIII. (May 14th, 8 A.M.), which had been under observation for three previous days, and not used in any other experiment. From the importance of the case, we think it well to give in addition to the temp. curve (Table V., curve XIII.) the table of figures.

TABLE VI.—*Temperature and Weight of No. XIII.*

Day.	Hour.	Temp.	Weight.	Day.	Hour.	Temp.	Weight.	Observations.
May 12	4 p.m.	39·5	1,567gr.	May 13	10 p.m.	39·65		
" "	6 "	39·35		" 14	6 a.m.	39 6		
" "	8 "	39·3		" "	8 "	39 5	1,481gr.	Injection.
" "	10 "	39·2		" "	10 "	40·5		
" 13	6 a.m.	39·3		" "	12 noon	40·1		
" "	8 "	39·7		" "	2 p.m.	41·1		
" "	10 "	39·3		" "	4 "	41·5		
" "	12 noon	39·5		" "	6 "	41·2		
" "	2 p.m.	39 9		" "	8 "	41·15		
" "	4 "	39·7		" "	10 "	41·2		
" "	6 "	39·3		" 15	6 a.m.	37·4		
" "	8 "	39·8		" "	8 "	—	—	Found dead.

* It is important to notice that the greatest malarious infections (XII., XIII., and XIV.) were produced by soil which had for three days been subjected to treatment, which, as we have said already, *impedes the development of algæ*, and arrests it at the outset in mud, in which it was most luxuriant, a fact which has an important bearing on the observations of Salisbury and Balestra.

Autopsy made immediately. Spleen much enlarged, soft, dark-coloured (Table I., fig. 13); L. 58·0; B. 14·3; T. 4·8. Having omitted to weigh the animal before opening it, the I. S. was calculated in reference to the animal's weight before the febrile *accès*. It was 2,124; *therefore it had become* 3·2 *times larger than the normal in* 24 *hours*.

The splenic pulp examined fresh contained *granules and irregular masses of brown and black pigment, very many automatically moving spores, some bacilli and long homogeneous filaments of a breadth of* 0·0006 *mm. and a mean length of* 0·07 *mm.* In the marrow of the bone were found many oval corpuscles, some of them enclosed in the large white cells, some of those free were double. Some self-moving oval bodies found in the blood of the portal vein and inferior vena cava.

In the lymph of the superior mesenteric glands were found very many oval spores and bacilli in abundance.

No other change was found in the other organs of the body. The gravity of this case, in which, without any complication, a perniciosa even more intense than that which killed No. XII. with identical microscopic results, renders very interesting the comparison of it with No. VIII. In the latter the same substance was injected in equal quantity to that used for No. XII., and double that used for No. XIII., but the liquid was first filtered through paper, and this simple and incomplete filtration was sufficient to reduce the morbid power from the proportions it assumed in Nos. XII. and XIII. to the slender ones even in No. VIII.

The third sample of soil, taken from a field recently ploughed in the Val de Cremera, was derived from a non-marshy soil, which owing to the ploughing had been exposed to the action of air more than the two preceding. This circumstance made us suppose that the development of malaria might here have attained greater proportions, and this was borne out by the results.

No. XIV. not used before, and whose temperature had been normal for two whole days, was injected May 14th, at 10 A.M., with 6·4 cc. of a mixture of equal parts of Marcia water and the soil in question, after subsidence of the heavier particles.

Table VII.—*Temperatures and Weights of No. XIV.*

Day.	Hour.	Temp.	Weight in Grammes.	Day.	Hour.	Temp.	Weight in Grammes.
May 12	4 p.m.	39·3	1,421	May 16	10 p.m.	40·5	
,, ,,	6 ,,	39·3		,, 17	6 a.m.	40·7	
,, ,,	8 ,,	39·3		,, ,,	8 ,,	40·8	
,, ,,	10 ,,	39·25		,, ,,	10 ,,	40·6	1,309
,, 13	6 a.m.	39·25		,, ,,	12 ,,	40·8	
,, ,,	8 ,,	39·2		,, ,,	2 p.m.	40·7	
,, ,,	10 ,,	39·0		,, ,,	4 ,,	40·6	
,, ,,	12 noon	39·1		,, ,,	6 ,,	40·7	
,, ,,	2 p.m.	39·4		,, ,,	8 ,,	41·1	
,, ,,	4 ,,	39·5		,, ,,	10 ,,	41·3	
,, ,,	6 ,,	39·3		,, 18	6 a.m.	41·1	
,, ,,	8 ,,	39·6		,, ,,	8 ,,	41·2	
,, ,,	10 ,,	39·5		,, ,,	10 ,,	40·9	
,, 14	6 a.m.	39·4	1,412	,, ,,	12 noon	40·7	
,, ,,	8 ,,	39·4 *		,, ,,	2 p.m.	40·6	
,, ,,	10 ,,	40·2		,, ,,	4 ,,	40·7	
,, ,,	12 ,,	39·9		,, ,,	6 ,,	40·75	
,, ,,	2 p.m.	41·25		,, ,,	8 ,,	40·5	
,, ,,	3 ,,	41·8		,, ,,	10 ,,	40·4	
,, ,,	6 ,,	41·3		,, 19	6 a.m.	40·4	
,, ,,	8 ,,	41·0		,, ,,	8 ,,	40·4	
,, ,,	10 ,,	40·9		,, ,,	10 ,,	40·5	
,, 15	6 a.m.	40·7		,, ,,	12 noon	40·4	
,, ,,	8 ,,	40·6		,, ,,	4 p.m.	40·3	
,, ,,	12 noon	40·4		,, ,,	6 ,,	41·2	
,, ,,	2 p.m.	40·8		,, ,,	8 ,,	41·5	
,, ,,	4 ,,	40·0		,, ,,	10 ,,	41·2	
,, ,,	6 ,,	40·7		,, 20	6 a.m.	40·7	
,, ,,	8 ,,	40·4		,, ,,	8 ,,	40·6	1,345
,, ,,	10 ,,	40·6		,, ,,	12 ,,	40·1	
,, 16	6 a.m.	40·1		,, ,,	2 p.m.	40·5	
,, ,,	8 ,,	40·0		,, ,,	4 ,,	40·2	
,, ,,	10 ,,	39·8	1,303	,, ,,	6 ,,	40·2	
,, ,,	2 p.m.	40·95		,, ,,	8 ,,	40·0	
,, ,,	4 ,,	40·2		,, ,,	10 ,,	40·5	
,, ,,	6 ,,	40·1		,, 21	6 a.m.	40·9 †	1,373
,, ,,	8 ,,	40·6					

* Injection, 6·4 cc. of liquid. † Was killed.

This fever is one of the most characteristic in its course, and at the same time one of the gravest, produced by us, having a maximum temp. in the first F. A. of 41°·8, and in the third of 41°·5. And although it was so grave, the weight went on increasing after the first remission, but not much (70 gr.)

Autopsy.—Great poverty of blood in the entire organism, and this so pale that it could not be used to obtain the nature print of the spleen, so that the blood of No. X., killed the same time, had to be used instead. Spleen very large, soft, dark-coloured, triangular on section (Table I., fig. 14). L. 62·4; B. 12·8; T. 4·8; I. S. 2,793, *i.e.*, four times the normal; contained large

quantity of black pigment and self-moving oval spores, diameter 0·00095, but no bacillus or filament.

The oval corpuscles also existed in the marrow and aqueous humour of the eye, reserved for microscopic use to dilute other preparations.

The lymph was very rich in these oval bodies, of the superior mesenteric glands, which was charged with pigment. In classifying these soils according to their infective power, the lowest place must be assigned to that from the marsh near the hamlet, and this and the soil from the garden of the Villa Spada seems to indicate that culture and admixture of excrementitious matter diminish the infective energy. We therefore made another experiment with soil from an artichoke ground near the ploughed field of which the soil was used in No. XIV. Time not permitting the employment of the means used in that case, we contented ourselves with mixing it with an equal quantity of water, and used the liquid after subsidence of the heavier particles. Nos. XV. and XVI. (not used before, and of which the temp. had been normal for two days) were injected with 6·4 cc. of the turbid unfiltered liquid in the case of No. XVI., and of the same after passing through a double filter in the case of No. XV. (Table V., curves XV. and XVI.)

No. XVI. had three febrile *accès* in three days, with always diminishing maxima and increasing minima. The type being quotidian passing into subcontinuous remittent. No. XV. had one *accès* of 40°·15 of short duration, then for 14 hours a subnormal temp. Afterwards three slight rises of 0°·1 or 0°·2 above the mean at irregular intervals.

This double experiment, while showing that the infective power of the liquid was diminished by filtration, also showed that it had been already much limited even before filtration, which may be cited in favour of the hypothesis, *that a judicious cultivation of the soil diminished the production of malaria, even in regions in which this production is greatest.*

We have the intention to undertake much more extensive and complete researches to endeavour to solve this problem.

Group V. *Experiments with specimens of earth derived from non-malarious places.*—We only had time for two experiments of this kind. It is very probable that the diffusion of these germs is greater than may be revealed by the existence of

endemic malaria; and that they exist in many places where this is lacking, simply because the necessary conditions of complete development are not present. By artificially procuring these conditions, we may render infective samples of earth from places where malaria shows no influence on the human body.

These two experiments were made with soil from a garden attached to the Pathological Institute of the University of Prague. This earth was a disintegrated silurian schist, *in situ* for twenty years, manured for the two last years, but not touched this year, and therefore very compact.

With this soil an artificial marsh was made as before, and kept for four days at a temp. of 35°—40°. June 9th, a portion of this was mixed with water, in the usual way, and 10 cc. of the turbid liquid injected under the skin of the back of a large rabbit, No. XIX., weighing 2,479 gr.

The liquid was then passed through a double filter of Swedish paper, and 10 cc. injected in the same way into No. XX., which weighed 1,680 gr.

After the injection there arose in No. XIX. a fever, during which the temp. was much above the normal (Table V., c. XIX. and XX.). This continuous fever had after the first remission (39°·9) two recrudescences, during which the temp. ran up to 41°·1 and 41°·8. From the last up to its natural death, which happened when the animal had still a temp. of 40°·4, elapsed 22 hours.

In No. XX. after injection arose a fever, similar to a quotidian. The six accesses are divided into two groups of three each, separated by an intermission between the 3rd and 4th *accès*, in which the temp. fell from 40°·0 to 37°·5, to rise again during the 4th *accès* to 40°·3. No. XX. was killed when No. XIX. died, and the two autopsies made at once.

In No. XIX. a large abscess at injection site, grave pulmonary œdema; organs atrophied and poor in fat; spleen enlarged and soft.

In No. XX. general atrophy very pronounced; spleen very small. Both had lost weight largely.

WEIGHT.

	At commencement.	At the end.	Loss.
XIX.	2,749 gr.	2,285·5 gr.	463·5 gr.
XX.	1,680 „	1,175·5 „	504·5 „

SIZE OF SPLEEN.

	L.	B.	T.	I. S.	
XIX.	60·8 mm.	11·2 mm.	4·0 mm.	1,261	Normal Index, 664.
XX.	30·8 „	4·8 „	1·6 „	201	

In No. XIX. the fever was undoubtedly septic. In No. XX. probably hectic from septic infection, in which, as often happens in man, the temp. alternations resemble malarious quotidian.

Group VI. *Septic infections produced spontaneously in animals under experiment.*—In two animals introduced into the laboratory septic infection occurred before they had been used for experiment. In one, No. VI., the temp., at first subnormal, increased continuously till it reached, before the natural death of the animal (Table IV., curve VI.), the height of 40°·9. At the autopsy was found grave peritonitis with effusion of serum and masses of fibrin, produced by perforating ulcer of the large intestine; anæmia, with dense grumous clots in the heart; lungs extremely œdematous; spleen moderately enlarged, soft, pale red coloured, angles much rounded (Table I., fig. 6). It contained no pigment.

No.	Weight of Body.		Dimensions of Spleen.			I. S.
	Beginning.	End.	Length.	Breadth.	Thickness.	
VI.	1,136 gr.	1,057 gr.	53·00 mm.	9·0 mm.	4·6 mm.	1968
	Loss ...... 79 gr.					

The second was a large rabbit (No. XI.), which had fever when brought to the laboratory (Table IV., curve VI.). The temp. rose on the second and third days to 40°·0 and 40°·3, then came two short remissions; the fourth day it rose gradually to 40°·8. Abscess in neck opened. Gradual fall of temperature for two following days, perhaps helped by magnes. benzoate. Strong fever lasting six and a half days, until it was killed. Fever always high even in the remissions, except at the middle of the second day, when for a very short time it was subnormal. This animal during the fever ate enormously and gained weight a little.

Autopsy.—Spleen very large and soft, with rounded borders and elliptical section (Table I., XI.); no pigment; blood watery and colourless; pleura pulmonalis ecchymosed; lungs hyperæmic and œdematous; right heart gorged, left empty; an immense abscess in submaxillary, and extensive purulent infiltration in scapular region.

No.	Weight of Body.		Dimensions of Spleen.			I. S.
	Beginning.	End.	Length.	Breadth.	Thickness.	
XI.	2,578 gr.	2,655 gr.	79 mm.	13·8 mm.	6·5 mm.	2669
	Gain......	77 gr.				

Supplementary Notes.

While the authors are aware that further and very extensive researches are required before the subject is exhausted, they claim to have proved—1st. *That malarious affections may be produced artificially in animals in the identical forms known to human pathology.* 2nd. *That these artificially-produced malarious affections are excited by organisms, which are found in the soil of malarious places before the appearance of the fevers, and are, even then, diffused in the strata of the air nearest the soil.*

Certain further facts, not fully set forth in the preceding, the confirmation of which was independently arrived at by Professor Marchiafava, call for statement.

1st. *Anatomical changes observed in animals in which malarious infection had been induced.*

a. The uniform swelling, triangular section, sharp edges and borders, markedly distinguish malarious from septic enlargement, in which the organ is rounded.

b. The presence of black pigment, derived, as Marchiafava has shown, from the hæmoglobin of the blood, and containing iron in inorganic combination, as shown by the blue colour resulting from the reaction with weak hydrochloric acid and ferrocyanide of potassium. *Therefore it is indisputable that in malarious diseases hæmoglobin becomes decomposed even in the blood globules still intact, in such a way as to set free the iron which at first was united with other organic compounds, and could not be traced by this reaction.* The name *melanæmic*, by which it is distinguished, is therefore justified. In the greater part of our artificially produced cases this pigment was present. The largest quantity was seen in the spleen of No. XIV., in which recently ploughed earth of Valchetta was used, and in which severe fever occurred running up to 41°·8. The pigment was most abundant

in the peripheral parts of the spleen, and formed masses, large and irregular, or of a rounded contour, slightly smaller than a blood globule. The transformation of the hæmoglobin was, in the preparations, traced through all its stages, and also in those preserved in Canada balsam.

In No. XIV. only was melanæmic pigment found in any other organ but the spleen. Perhaps the brief duration of very severe fever in this case, and the slight gravity but long continuance of it in the others, explains this difference. Even in this animal, however, the pigment could not be seen except in the superior mesenteric glands, which were of a greyish brown colour. The pigment was chiefly accumulated in the lymph sinuses, where groups of granules were seen, some transparent and of a nickel colour, others opaque and black.

The perfect identity of these with those found in the spleen, the smallness of the pigment masses, the absence of transition forms, and the site of the accumulations, show that the pigment had not been formed in those glands, but conveyed to them after the disintegration of the pigment masses. Since neither the blood nor inferior lymphatic glands contain traces of this pigment, it cannot have been derived from the intestines or blood vessels, but must be supposed to have been imported into the superior lymphatic vessels by the lymphatics which connect them with the spleen.

In Nos. XII. and XIII. the amount of pigment was much less than in No. XIV. Of the two former, the spleen of No. XIII. was richest in melanæmic pigment; although the duration of fever was shorter, the temperature and swiftly fatal course point to a severer form of infection than in No. XII. The greater proportion was found in the Malpighian corpuscles and in the vicinity of the arteries, probably owing to rapid introduction of pigment into the circulation, as we cannot admit that it had formed in these corpuscles, in which we never found any trace of pigmentation, not even in No. XIV., which had a *true black* spleen.

The two cases of septicæmia are directly opposite in this respect. In neither was any black, or even brown, pigment found in the spleen, but only large white cells full of small granules of an uniformly orange colour.

2. *Bacillus malariæ.*—We have described in Chapter III. the

principal forms of the plant found in the soil and air of malarious places, which, after cultivation, produced forms of disease exactly the same as those produced by the crude material which was the basis of cultivation; and it is sufficient to have shown experimentally that one determinate form of bacillus must be regarded as the exciting cause of the disease. It remains for botanists to determine what ulterior developments this organism may have, and all its biological attributes. We have stated how the commoner forms of *bacillus malariæ* proceed from homogeneous filaments often twisted or looped, which, when cultivated in fish gelatine, in egg albumen, urine, divide into joints, and produce spores in their interior both before and after this division. The situation of the spores varies; sometimes they are only found at the two ends of the articulation, at other times in the middle, and again at another time there may be a median spore and also two terminal ones—characteristics which distinguish *bacillus malariæ* from all other bacilli.

Some cultivations showed us very well the development of filaments from these spores when set free. In the oval shining corpuscles so often described, one of the poles is elongated into an appendix, which is slowly converted into a rod; sometimes in young spores one sees the clearing up of the substance of one pole precede the formation of the appendix; at other times the formation proceeds from both poles of the spore at the same time, leading to the formation of two appendices.

The rods elongating produce homogeneous filaments; sometimes two terminal spores are developed in the interior of these, or one median; in a few cases the spores are developed in their interior in such a way as to make one think that they are the products of the division *in situ* of a previously formed spore. Sometimes before the production of articulated filaments we see form in their interior some brilliant oval bodies (Table II., fig. 1, *d*), and the filament grows so rapidly that one can watch its increase with the eye, becoming full of small brilliant granules, whilst the oval spores fade away.

We are not in a position to decide whether the small granules are derived from the breaking up of the spores, or a multiplication of them due to a new precipitation of plasma.

This mode of evolution seems to be connected with certain forms seen by us in cultivation of the air of Ninfa and Fogliano

in urine, and of Caprolace mud in fish jelly. They are shown in fig. 8, Table II., as seen with 1-12 in. objective (oil immersion), and No. IV. eyepiece of Zeiss—jointed filaments, full of small shining granules (*a*, *a*, *a*). The division into joints commences in these filaments with the formation of a dividing membrane which separates the two masses of protoplasm. The extremities of these newly-formed joints are rounded, the distance between two contiguous joints becomes greater, and the uniting band so weak as to be readily severed. The granular homogeneous masses contained in the joints divide longitudinally or transversely, or both, producing spherules separated by clear spaces.

We are unable to say whether these forms belong to the normal evolution of this bacillus, or represent anomalies produced by the special accidents of our cultivation. The sum of our observations leads us to think that the forms of fig. 8 belong to this species of bacillus. Should further observations show the contrary, that would not change our conclusions, because in such a case we should regard it as an admixture with other forms of schistomycetes.

Fig. 5, Table II., shows exactly the advanced development of the plant seen in the serum of the lymph of No. I. This was enclosed, April 21st, in a microscopic air-cell. It contained oval self-moving bodies, some of which had two polar appendices, and some homogeneous, slightly oscillating filaments. Next day were found the forms of (fig. 4, Table II.), viz., free spores, twisted filaments, some of which had spores at the two ends.

April 23rd the development of filaments was enormously increased, the bundles forming a network.

So that from a liquid of the body of an infected animal were obtained, without the addition of any other substance, forms identical with those obtained by means of the first cultivation of the crude material and subsequent cultivations of the same.

Long homogeneous filaments, identical with those obtained by cultivations, were met with in the spleen of No. XIII., and in the spleen and bone marrow of No. XII.

3. *Bacillus malariæ in man.*

Dr E. Marchiafava, first assistant to the Professor of Pathological Anatomy in the University of Rome, had an opportunity

of examining the bodies of three persons very recently dead of perniciosa. The autopsies were made with the utmost care to avoid sources of error. In the first, made July 25th, the examination of the blood was omitted. In the spleen and bone medulla no bacillary forms were met with, but only a large quantity of spores.

The second was made August 19th, on a man who died in the second *accès* of *perniciosa comatosa*, in the Santo Spirito Hospital. The spleen was doubled in size, brown, and very soft; the bone marrow brownish-red; the kidneys, liver, brain and its membranes rich in blood; the right heart full of fluid blood, the left empty; bilateral hypostatic congestion of lungs. No other change. The splenic pulp, juice of bone marrow, and of cæliac lymphatic glands, the blood of the splenic vein, hepatic veins, portal vein, and of the right heart, collected in capillary tubes, and sealed with the lamp. In the splenic pulp and bone marrow a small quantity of rusty pigment was found in free masses, or inclosed in the cell elements, a distinct quantity of oval spores, and some rods like those in fig. 1, *a*, Table II. In the lymphatic gland juice and in all the samples of blood were found a great quantity of bacilli, some homogeneous, others jointed and perfectly identical with those in fig. 3, Table II. Cultivation of splenic pulp and a few drops of blood in urine, in the usual way produced forms identical with figs. 5 and 6, Table III. In the third case, similar in every respect, there was in addition very grave general melanæmia; spleen doubled in size; pulp diffluent and black; bone marrow chocolate colour; liver lead colour, and grey substance of the brain slate colour. In the blood and throughout the body pigment masses free and inclosed in the white cells. In the blood, marrow, and spleen, very numerous oval spores, endowed with very active movements. In addition, in the spleen and blood oscillating rods like fig. 1, *a*, Table II., and nucleated bacteria like fig. 1, *f*, and fig. 7, *c*.

In the blood, everywhere, and in the spleen and marrow a very large number of long homogeneous filaments, some twisted like fig. 7, Table II., and long homogeneous and jointed filaments perfectly identical with fig. 5.

Dr. Valenti, Professor of General Pathology in the University of Rome, took part in some of these observations, and the

results of the last autopsy were verified by Professor Tommasi-Crudeli.

NOTICE TO THE READER.

The original of this Paper is profusely illustrated by plates and temperature charts. The reproduction of these would have involved too great expense, and they have therefore been omitted. The references have, however, been retained in the translation as being useful to those readers who may wish to pursue the subject in more detail in the Author's own words.

The same statement applies also to the following Paper.